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(54) Title: A MODIFIED THERMO RESISTANT DNA POLYMERASE

(57) Abstract

Novel, modified Taq DNA polymerases and genes encoding for them are disclosed. The modified Taq DNA polymerases of the invention are the same size, have the same heat stability and synthesis rate as the native enzyme, but lack the 5'-3' exonuclease activity. As a result of this modification, the enzymes have improved processivity as compared to the native enzyme. The enzymes of the present invention enable improved methods of conducting PCR, DNA sequencing, and DNA synthesis.

Scheme for Zone Mutagenesis

Eco RI Kpnl BstXI

PUC18

Mutagenesis Trestments 1-4

PCR

PCR

Rt Kpnl Rt BstXI

(REM-T2)

Ligate

Trunsform and Assay

Identification of REM-T3

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MODIFIED THERMO-RESISTANT DNA POLYMERASES

BACKGROUND OF THE INVENTION

"This invention was made with Government support under grant GM 24711 awarded by the National Institutes of Health. The Government has certain rights in the invention."

Field of the Invention

This invention relates to the field of DNA polymerases for use in the polymerase chain reaction and DNA sequencing.

Description of the Prior Art

Polymerase Chain Reaction (PCR) was one of the most important inventions developed in area of biotechnology during the 1980's and has proven useful for a variety of tasks. PCR Technology, Principles and Applications for DNA Amplification (Erlich ed. 1989). The process provides a method for amplifying known specific nucleic acid sequence. Mullis, U.S. Pat. No. 4,683,202. The process comprises treating single or double stranded DNA containing the sequence of interest with an excess of two oligonucleotide primers sufficiently complementary of the strands so as to hybridize to the denatured strands. The hybridized primers are then extended by a DNA polymerase in the presence of the four dNTPs. The primer extension products are then separated and can serve as templates for another cycle of replication. The number of DNA templates approximately doubles on each cycle of amplification. Thus, 20 cycles of the process will result in approximately a 220-fold amplification.

The original protocols for PCR used the Klenow fragment of E. coli DNA polymerase I to catalyze the extension of the oligonucleotide primers. Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51, 263 (1986); Mullis and Faloona, Methods

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Enzymol. 155, 335 (1987). The Klenow fragment proved somewhat cumbersome to use. Denaturation of the double stranded DNA at the start of each cycle requires temperatures ranging from 80 to 105°C. These temperatures inactivate the Klenow fragment. Consequently, fresh enzyme was required at the start of each new amplification cycle. While this process generally worked well for small segments of DNA (< 200 bp), a host of problems arose when replication of larger fragments was attempted.

The difficulties associated with use of the Klenow fragment DNA polymerase were circumvented with the introduction of thermostable DNA polymerase obtained from the thermophilic bacterium *Thermus aquaticus* (*Taq* DNA polymerase). Saiki et al., *Science* 239, 487 (1989); Gelfand et al., U.S. Pat. No. 4,889,818. This enzyme has been cloned, overproduced, and the DNA sequence determined. Lawyer et al., *J. Biol. Chem.* 264, 6427-6437 (1989).

In addition to its DNA polymerase activity, Taq DNA polymerase also possesses 5 '-3' polymerization-dependent exonuclease activity, but it lacks 3 '-5' exonuclease activity. Longley et al., Nuc. Acids Res. 18, 7317-7322 (1990); Blanco et al., Gene 100, 27-38 (1991); Bernad et al., Cell 59, 219-228 (1989); Lawyer et al., supra; Holland et al., Proc. Natl Acad. Sci. 88, 7276-7280 (1991); and Kelly and Joyce, J. Mol. Biol. 164, 529-560 (1983). Studies have identified the 5'-3' exonuclease activity as being an intrinsic part of Taq DNA polymerase. Longely et al., supra; and Barnes et al., Gene 112, 29-35 (1992). This activity appears to facilitate a nick translation DNA reaction.

Native Taq DNA polymerase suffers from a high rate of misincorporation — about four times higher than that of the Klenow fragment of E. coli DNA polymerase I.

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It has been estimated that *Taq* DNA polymerase incorporates one incorrect nucleotide in 9000. Tindall and Kunkel, *Biochemistry* 27, 6008 (1988). After 20 amplification cycles, this would result in DNA molecules with random mutations averaging one in every 900 bases. Saiki et al., *supra*. If the PCR product is to be inserted into an expression vector, the chance that one cloned molecule will contain an unwanted sequence alteration may be significant. It would be desirable, therefore, to decrease the rate of misincorporation of the DNA polymerase used in PCR without sacrificing the heat stability and rate of synthesis of the native *Taq* DNA polymerase.

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It has been shown that removal of the 5'-235 codons of the Taq DNA polymerase gene results in an expression product that has no 5'-3' exonuclease activity and a lower rate of mutagenesis. Tindall et al., supra; and Barnes, supra.

Other forms of *Taq* DNA polymerase are available. AmpliTaqTM is a commercially available genetically engineered version of *Taq* DNA polymerase and is substantially equivalent to the native form. Perkin Elmer Cetus; Saiki and Gelfand, *Amplifications* (Perkin Elmer Cetus), 1, 4 (1989). Also commercially available is a truncated gene product, the Stoffel fragment, that expresses an enzyme lacking the 5 '-3' exonuclease activity and having much lower unit activity, probably due to decreased processivity and increased mutagenesis. Barnes, *supra*. Gelfand and Abramson (PCT International Publication No. WO 92/06200) disclosed a modified *Taq* polymerase having the same length as the native enzyme, but with highly attenuated 5 '-3' exonuclease activity. The exonuclease activity is defeated by mutation in nucleotide 137 of the *Taq* polymerase gene, wherein the mutation is G

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to A, resulting in a change in amino acid 46 of the enzyme from Gly to Asp. This enzyme is reported as having the same polymerase activity, processivity and extension rate as the native enzyme.

SUMMARY OF THE INVENTION

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An object of this invention is to enhance the synthesis activity of DNA polymerase as used in PCR and DNA sequencing.

The invention disclosed herein achieves this object by providing a modified *Taq* DNA polymerase and a correspondingly modified *Taq* DNA polymerase gene sequence. The modified *Taq* DNA polymerase is the same size, has the same heat stability and synthesis rate as the native enzyme, but the 5 '-3' exonuclease activity is missing. As a result of this modification, the gene expression product has improved processivity.

The enzymes of the present invention enable improved methods of conducting PCR, DNA sequencing and DNA synthesis.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphical depiction of the restriction map of the *Taq* DNA polymerase gene.

Figure 2 is a graphical depiction of the method for producing the modified Taq DNA polymerase and the gene encoding it.

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Figure 3 shows the sequencing primers for the pLSM5 (SEQ ID NO: 3) plasmid.

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Figure 4 is a schematic depiction of the method for testing processivity used in trials 1 and 2.

Figure 5 is the autoradiograph showing the results of processivity testing used in trial 1.

Figure 6 is the autoradiograph showing the results of processivity testing used in trial 2.

Figure 7 is a schematic depiction of the method for testing processivity using PCR.

Figure 8 is the autoradiograph showing the results of processivity testing by the PCR method.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "replication product" refers to the oligonucleotides synthesized by DNA polymerase, whether it be as part of the polymerase chain reaction, DNA sequencing, or any other reaction where DNA polymerase is used to synthesize an oligonucleotide.

The term "oligonucleotide" as used herein is defined as a molecule composed of two or more deoxyribonucleotides or ribonucleotides.

The term "thermostable" refers to an enzyme that is stable to heat (> 95°C) and catalyzes combination of nucleotides to form an oligonucleotide. The term "thermo stability" as used herein refers to the characteristic stability of an enzyme to heat.

As used herein, the term "altered amino acid" means an amino acid that differs from that found in the native peptide or protein. Hence, if the native peptide

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has the amino acid Cys at position 43, and the modified peptide has the amino acid Gly at that position, Gly is the "altered amino acid." Similarly, the term "altered nucleotide" means a nucleotide that differs from that found in a native oligonucleotide, polynucleotide, gene, or other nucleotide fragment.

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As used herein, the phrase "lacking 5'-3' exonuclease activity" means an enzyme having less than 1% of the 5'-3' exonuclease activity of the native Taq DNA polymerase.

We undertook to inactivate the 5 '-3' exonuclease activity of the *Taq* DNA polymerase by *in vitro* mutagenesis without removal of the portion of the gene encoding that activity. The procedure followed was to develop a method of "zone mutagenesis" for that region of the *Taq* DNA polymerase gene encoding for the 5 '-3' exonuclease activity. *See* Figure 2. The nucleic acids encoding the amino acid residues required for 5 '-3' exonuclease activity have not been clearly identified, but earlier work suggests the region involved in DNA polymerases from other bacteria. Kelly and Joyce, *supra*.

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To briefly summarize, using PCR technology we generated a *Taq* gene, which we cloned into the plasmid vector pUC18. *See* Figure 1. The pUC18 plasmid containing the *Taq* gene is designated pLSM5 (SEQ ID NO: 3). There are four base changes in the *Taq* gene produced by PCR and cloned in pLSM5 (SEQ ID NO: 3) compared to the published *Taq* DNA polymerase gene sequence (TTHTAQPIA in GenBank) (SEQ ID NO: 1): 1) C to G at position 89 in the untranslated 5 end, 2) T to A at position 934 (Phe to Ile), 3) T to C at position 962 (Leu to Pro), and 4) G to A (no amino acid change) at position 2536. The protein expression product of this

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gene has an altered amino acid at positions 272 (IIe) and 281 (Pro). We then subjected the pLSM5 (SEQ ID NO: 3) plasmid to conditions that would cause the random mutations in the 5° exonuclease domain.

The vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) begins at nucleotide 70 and ends at 2619. The reading frame for translation begins at nucleotide 121 and ends at 2619 by the convention of Lawyer et al., *J. Biol. Chem.* 264, 6427-6437 (1989).

The following sequence appears at the 5° junction between the pUC18 plasmid and the Taq gene:

... AATTTCACACAGGAAACAGCTATGACCATGATTACG<u>AA</u>TTCTAAA ... (SEQ ID NO: 14)

This sequence begins with the pUC18 antisense nucleotide sequence 490 to 455. The underlined nucleotides (AA) were added to create a restriction site. The *Taq* gene sequence (bold face) begins at nucleotide 70).

The following sequence appears at the 3 junction between the pUC18 plasmid and the Taq gene:

....CAAGGAGTGAGATTCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTT ... (SEQ ID NO: 15)

This sequence begins with *Taq* polymerase gene nucleotide 2610 to 2619. The underlined nucleotides (GA) were added to create a restriction site. The remaining sequence is the pUC18 antisense nucleotide, 413 to 381. Both junction sequences have been verified by sequence analysis.

The enzyme expression product of the pLSM5 plasmid, REM-T2 (SEQ ID NO: 4), has substantially the same processivity, 5 '-3' exonuclease activity, and

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performance in normal PCR, to the extent tested so far, as the commercially available Taq DNA polymerase AmpliTaqTM.

A variety of methods of mutagenesis are known to those of skill in the art and may be used in preparing a modified Taq DNA polymerase gene according to the present invention. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 2d Ed. 1989). The mutated genes were then treated with restriction endonucleases that cut it in the region believed to be responsible for 5 '-3' exonuclease activity, thereby producing mutated inserts coding for that portion of the gene. A vector containing the native Taq DNA polymerase gene was treated with the same endonucleases and the previously obtained inserts ligated into the vector. Cells were transformed with the vector containing the inserts and colonies grown. We assayed polymerases expressed by the various colonies for polymerase activity as well as 5'-3' exonuclease activity. The cells transfected with the gene encoding the modified Taq DNA polymerase meeting the objective of the present invention were thereby identified.

Appropriate host cells for the present invention may be chosen from the prokaryote group, which most frequently are represented by various strains of *E. coli*. Other microbial strains such as bacilli may be used, however. *Bacillus subtilis* and various species of *Pseudomonas* may be used, for example. In such prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host are used. For example, *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species by Bolivar, et al., *Gene* 2, 95 (1977). pBR322 contains genes for ampicillin and tetra-

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cycline resistance, and thus provides addition markers that can be either retained or destroyed in constructing the desired vector. Commonly used prokaryotic control sequences, which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequence, include such commonly used promoters as the β -lactamase (penicillinase) and lactose (lac) promoter systems (Chang, et al., Nature 198, 1056 (1977)), the tryptophan (trp) promoter system (Goeddel, et al., Nucleic Acids Res. 8, 4057 (1980)), the lambdaderived P_L promoter (Shimatake et al., Nature 292, 129 (1981)), and the N-gene ribosome binding site, which has been made useful as a portable control cassette (U.S. Pat. No. 4,711,845). The N-gene ribosome binding site comprises a first DNA sequence that is the P_L promoter operably linked to a second DNA sequence corresponding to N_{RBS} upstream of a third DNA sequence having at least one restriction site that permits cleavage within six bp 3 of the N_{RBS} sequence. Also useful is the phosphatase A (phoA) system described by Chang et al. in European Patent Publication No. 196,864 published Oct. 8, 1986. Any available promoter system compatible with prokaryotes can be used, however.

In addition to bacteria, eucaryotic microbes, such as yeast, may also be used as hosts. Laboratory strains of Saccharomyces cerevisiae, Baker's yeast, are most used, although a number of other strains are commonly available. While vectors employing the 2 micron origin of replication are illustrated (Brach, Meth. Enz. 101, 307 (1983)), other plasmid vectors suitable for yeast expression are known (see, e.g., Stinchcomb et al., Nature 282, 39 (1979), Tschempe et al., Gene 10, 157 (1980), and Clarke et al., Meth. Enz. 101, 300 (1983). Control sequences for yeast vectors include promoters

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for the synthesis of glycolytic enzymes. Hess et al., J. Adv. Enzyme Reg. 7, 149 (1968) and Holland et al., Biotechnology 17, 4900 (1978).

Additional promoters known in the art include the promoter for 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255, 2073 (1980) and those for other glycolytic enzymes, such as glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other promoters that have the additional advantage of transcription controlled by growth conditions are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and enzymes responsible for maltose and galactose utilization. Holland, *supra*.

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It is also believed that terminator sequences are desirable at the 3° end of the coding sequences. Such terminators are found in the 3° untranslated region following the coding sequences in yeast-derived genes. Many of the vectors illustrated contain control sequences derived from the enolase gene containing plasmid peno46 (Holland et al., *J. Biol. Chem.* 256, 1385 (1981) or the LEU2 gene obtained from YEp13 (Broach et al., *Gene* 8, 121 (1978). Any vector containing a yeast-compatible promoter, origin of replication, and other control sequence is suitable, however.

It is also possible to express genes encoding polypeptides in eucaryotic host cell cultures derived from multicellular organisms. *See, e.g., Tissue Culture* (Cruz and Patterson eds., Academic Press 1973). Useful host cell lines include murine myelomas N51, VERO and HeLa cells, and Chinese Hamster Ovary (CHO) cells.

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Expression vectors for such cells ordinarily include promoters and control sequences compatible with mammalian cells such as the commonly used early and late promoters from Simian Virus 40 (SV 40) (Fiers et al., *Nature* 273, 113 (1978)) or other viral promoters such as those derived from polyoma, Adenovirus 2, bovine papilloma virus, or avian sarcoma viruses, or immunoglobulin promoters and heat shock promoters. A system for expressing DNA in mammalian systems using the BPV as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. General aspects of mammalian cell host system transformations have been described by Axel, U.S. Pat. No. 4,399,216. It now appears that "enhancer" regions are important in optimizing expression. These generally are sequences found upstream of the promoter region. Origins of replication may be obtained from viral sources. Integration into the chromosome, however, is a common mechanism for DNA replication in eucaryotes.

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Plant cells are also now available as hosts. Control sequences compatible with plant cells such as the nopaline synthase promoter and polyadenylation signal sequence are available. Depicker et al., J. Mol. Appl. Gen. 1, 561 (1982).

In addition, expression systems employing insect cells utilizing the control systems provided by baculovirus vectors have been described. Miller et al, Genetic Engineering 8, 277-297 (Setlow et al. eds. Plenum Publishing 1986). These systems are also successful in producing Taq DNA polymerase.

Cells transformed with the modified *Taq* DNA polymerase gene may be grown using any suitable technique. The appropriate technique will depend on the cell type and will be known to those skilled in the art.

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Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The treatment employing calcium chloride is used for prokaryotes or other cells that contain substantial cell wall barriers. Cohen, *Proc. Natl. Acad. Sci. (USA)* 69, 2110 (1972). Infection with *Agrobacterium tumefaciens* is used for certain plant cells. Shaw et al. *Gene* 23, 315 (1983). For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb is preferred. *Virology* 52, 546 (1978). Transformations into yeast are carried out according to the method of Van solingen et al., *J. Bact.* 130, 946 (1977) and Hsiao et al., *Proc. Natl. Acad. Sci. (USA)* 76, 3829 (1979).

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Construction of suitable vectors containing the desired coding and control sequences employs standard ligation and restriction techniques that are well understood in the art. Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and relegated in the form desired.

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Site-specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions that are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes. See, e.g., New England Biolabs, Product Catalog. In general, about 1 μ g of plasmid or DNA sequence is cleaved by one unit of enzyme in about 20 μ l of buffer solution. Often excess of restriction enzyme is used to ensure complete digestion of the DNA substrate. Incubation times of about one hour to two hours at about 37°C are workable, although variations are tolerable. After each incubation, protein is removed by extraction with phenol/chloroform, and may be followed by ether extraction. The nucleic acid may be recovered from

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aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations is found in *Methods in Enzymology* 65, 499-560 (1980).

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Cells producing enzyme of the desired type can be identified by standard techniques for assaying DNA polymerase and 5 '-3' exonuclease activity. *Id.* Using some of these methods, we were able to isolate a *Taq* DNA polymerase having the same size, heat stability, and synthetic activity of native *Taq* DNA polymerase, but having increased processivity and resulting in decreased mutagenesis of PCR DNA products. *See* examples *infra*.

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The modified *Taq* DNA polymerase of the present invention was chosen from a colony producing the enzyme with a relatively high polymerase activity and low 5 '-3' exonuclease activity. We designated this product REM-T3 (SEQ ID NO: 6). An equivalent independently isolated product with a different mutation but equivalent properties is designated REM-T5 (SEQ ID NO: 8).

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In addition to the modifications of native Taq DNA polymerase present in the modified Taq DNA polymerase of the present invention, individual amino acid residues in the peptide chain comprising the Taq DNA polymerase may be modified or deleted without eliminating any of the requisite properties described herein. Such alterations that do not destroy activity do not remove the DNA sequence or the modified Taq DNA polymerase from the contemplated scope of the present invention.

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In order to assay the modified Taq DNA polymerase, REM-T3 (SEQ ID NO: 6), it was necessary to isolate it. We used the following novel, short isolation technique producing high purity enzyme quickly. Bacteria were grown overnight or to an OD at 600 nm of about 2.0 to 2.5 and then centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the pellet washed with a solution of 50 mM Tris(8.0), 50 M dextrose, and 1 mM EDTA (15 x cell wt). The pellet was re suspended and lysed with a solution of 50 mM Tris, 50 mM dextrose, 1 mM EDTA, and 1 mg/ml lysozyme(5x cell wt). An equal volume of a solution of 10 mM Tris and 50 mM KCl, and 1 mM EDTA was added and the resulting mixture incubated at 75°C for 60 min before centrifuging at 8000 rpm for 15 min. The pellet was discarded and an equal volume of DEAE and 0.4 M KPO₄ (6.8) was added to the supernatant. The mixture was then incubated at 0°C for 30 min and then centrifuged at 10,000 rpm for 20 min. The pellet was discarded and the supernatant put on a phosphocellulose column with 0.02 M KPO₄ (7.5)(4x cell wt). The column was eluted with a gradient of 0.02 to 0.4 M KPO₄ (7.5). The peak was collected and applied to a Bio Rex-70 column with a solution of 0.02 M KPO₄ (7.6), 80 mM KCl 5%, glycerol, 0.5% Tween, and 0.5% Nonidet P-40. This column was then eluted with a step gradient of 0.3 M KCl and the peak collected.

The thermostability of the modified Taq DNA polymerase of the present invention must be substantially equivalent to that of native Taq DNA polymerase, i.e., it must not become irreversibly denatured (inactivated) when subjected to the elevated temperatures for the time necessary to effect denaturation of double-stranded nucleic acids. The heating conditions (e.g., temperature and time) necessary

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for denaturation will depend on a variety of factors, including the buffer salt concentration and the length and composition of the nucleotide chain. Typically, the temperature range for which the enzyme must be stable is about 90 to about 105°C for about 0.5 to four minutes. These values may vary depending on the conditions.

The modified *Taq* DNA polymerase of the present invention preferably functions optimally at temperatures above 40°C. The enzymes of the present invention is active in the temperature range 55 - 95°C, and preferably in the range 70 - 95°C.

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U.S. Pat. No. 4,889,818 discloses and claims a native form of *Taq* DNA polymerase. Because the modified *Taq* DNA polymerase of the present invention retains all the characteristics of the native form that are useful in PCR technology, its use in PCR is preferable to the native form. Consequently, applications using *Taq* DNA polymerase as described in U.S. Pat. No. 4,889,818, col. 14, 1. 33 to col. 27, l. 27 may also use the modified *Taq* DNA polymerase of the present invention. Accordingly, the disclosure of U.S. Pat. No. 4,889,818 is hereby incorporated by reference.

Besides use in the polymerase chain reaction, the modified *Taq* DNA polymerase of the present invention can be used in DNA sequencing by, for example, the Sanger dideoxy-mediated chain-termination method. Sanger et al., *Proc. Natl. Acad. Sci.* 74, 5463 (1977). Other similar uses will be known to those of skill in the art.

The following examples further elucidate the present invention, but are not intended to limit it.

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EXAMPLE 1

Zone Mutagenesis of the Taq DNA Polymerase Gene - Treatment 1

The *Taq* polymerase gene was amplified from genomic DNA (*Thermus aquaticus*) using primers adding an EcoRI site in the 5° UTR (nucleotide 70) and BgII site at the 3° end (nucleotide 2619). The the PCR product was cloned into pUC18 after digesting the vector with EcoRI and BamHI. *See* Figure 1. We designated this *Taq* gene REM-T2. We then incubated the plasmid containing the *Taq* gene at pH 4.8 (10 mM sodium acetate) and room temperature for 20 minutes followed by neutralization to pH 8.0 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)) followed by digestion with Eco RI and KpnI.

The pLSM5 (SEQ ID NO: 3) vector was digested with EcoRI and KpnI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5 '-3' exonuclease activity.

EXAMPLE 2

Zone Mutagenesis of the Taq DNA Polymerase Gene - Treatment 2

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. See Figure 1. We incubated the plasmid DNA containing the

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Taq gene at pH 4.8 and 60°C for 5 minutes followed by neutralization to pH 8.0 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11)) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)) followed by digestion with Eco RI and KpnI.

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and KpnI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5 '-3' exonuclease activity.

EXAMPLE 3

Zone Mutagenesis of the Taq DNA Polymerase Gene - Treatment 3

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. *See* Figure 1. We amplified the N-terminal region of the *Taq* DNA polymerase gene for three consecutive PCR programs of 30 cycles each using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)). Inserts for the putative amino terminal region of the gene were generated by digestion of the PCR products with Eco RI and KpnI.

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and KpnI and purified.

The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5 '-3' exonuclease activity.

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EXAMPLE 4

Zone Mutagenesis of the Taq DNA Polymerase Gene - Treatment 4

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. *See* Figure 1. We incubated the plasmid DNA containing the *Taq* gene a pH 4.8 and 70°C for 15 minutes followed by neutralization to pH 8 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11)) and the "sequencing primer" 1155A (CAG GTC CCT GAG GGC (SEQ ID NO: 13)) and 5x concentration of dNTPs (0.75 mM) followed by digestion with Eco RI and BstXI.

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A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and BstXI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5 '-3' exonuclease activity.

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EXAMPLE 5

DNA Polymerase Activity Assay

Assay mixture:

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reaction volume: 0.3 ml

25 mM Tris-HCl (pH = 8.8)

4 mM MgCl₂

22 μg activated ssDNA (salmon sperm)

0.033 mM dNTP (each)

2 μ Ci [methyl-³H] thymidine 5 'triphosphate

enzyme

Assay procedure:

The mixture was incubated at 75°C for 10 minutes. The reaction was stopped with 2 ml ice cold 10% TCA - 0.1 M sodium pyrophosphate. The tubes were then placed on ice for 10 minutes and the reaction volume filtered. The tube and filter were washed three times with 2 ml of 10% TCA - 0.1 M sodium pyrophosphate. The filter was then washed with 10 ml 0.01 N HCl. Next the filters were dried at 120°C for 15 minutes. The dried filters were counted in 1 ml of Scintiverse.

The results are displayed in Table 1, infra.

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EXAMPLE 6

5'-3' Exonuclease Activity Assay

Preparation of double stranded substrate with blunt ends and removal of 5° phosphate

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A Blue-Script plasmid was cut with HincII to produce one double stranded piece with blunt ends and treated with CIP (calf intestine phosphatase) to remove the 5° phosphate.

End-labeling of the 5 ends using [q-\|o(_,32)P]ATP

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8 μ l plasmid and 4 μ l buffer were mixed with spermidine and 28 μ l distilled H₂O. The mixture was then heated to 70°C for 5 minutes and then chilled on ice for 2 minutes. 10 μ l kinase buffer with 1 μ l [g-³²P]ATP (about 10 mCi) and 2 μ l (20 units) of T4 polynucleotide kinase were added. Then the mixture was incubated for 30 minutes at 37°C. The reaction was stopped by adding 2 μ l 0.5 M EDTA. The enzyme was inactivated by incubating for 10 minutes at 70°C. The radioactive ATP was removed by washing 4 times (2 ml each) in Centricon 100. The final volume was about 50 μ l (38,000 cpm/ μ l).

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5 - 3 exonuclease assay Assay conditions:

reaction volume 50 μ l

25 mM Tris HCl (8.8)

4 mM Mg Cl₂

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$0.5 - 1 \mu l$ labeled substrate

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0.3 units of DNA polymerase

Samples were incubated at 50 - 55°C for 15, 30 or 60 minutes. The reaction was stopped with 0.3 ml 10% TCA. The sample was microfuged for 15 minutes at 4°C. 0.1 ml was sampled on filter paper. The filter paper was dried at 120°C for 15 minutes. Dried filters were counted in 1 ml of Scintiverse.

The assay results are presented in Table 1, infra.

EXAMPLE 7

Sequencing Mutant Genes

Three mutants were chosen from those listed in Table 1 for low exonuclease activity. These were colony 18° (the plasmid of which we designate pTarf2 (SEQ ID NO: 9)) and colony 20° (the plasmid of which we designate pTarf3 (SEQ ID NO: 5)). A third mutant, pTarf5 (SEQ ID NO: 7), was obtained in a similar manner as in Example 4. pTarf3 (SEQ ID NO: 5) produces REM-T3 (SEQ ID NO: 6) and pTarf5 (SEQ ID NO: 7) produces REM-T5 (SEQ ID NO: 8). Bi-directional sequencing of the nucleic acid sequence of these mutants was conducted in the following manner: DNA sequence analysis was performed on alkaline-denatured double stranded plasmids. We used synthesized oligonucleotide primers (Fig. 3), [\$\alpha\$-\$\frac{35}{5}\$]-dATP, and Sequenase® T7 DNA polymerase kit (United States Biochemical Corp.) according to the manufacturer's conditions. This method is based on the dideoxy chain termination reaction (Sanger, Science 214, 1205 (1981).

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The alterations found in the mutants are presented in Table 2. These alterations are of the pLSM5 (SEQ ID NO: 3) sequence, i.e., the pTarf2 (SEQ ID NO: 9), pTarf3 (SEQ ID NO: 5), and pTarf5 (SEQ ID NO: 7) sequences are the same as the pLSM5 (SEQ ID NO: 3) sequence except for the alterations listed in Table 2.

TABLE 1

Enzyme Activity Of New Taq Clones

treatment	colony	polymerase act units/μl	5 '-3' exonuclease activity % of REM-T2 (SEQ ID NO: 4)
1	1	0.132	87
	2	0.503	97
	3	0.053	14
	4	0.27	88
	5	0.098	82
	6	0.41	94
	7	0.255	95
2	8	0.106	74
1	1.	1.54	104
	2-	1.60	94
	3 -	1.06	105
	4 -	1.49	100
	5.	1.06	104
	6.	2.20	114
	7.	0.35	107
	8.	0.68	117

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		9.	0.74	94
		10	0.87	109
	2	11 '	1.81	98
		12 '	1.22	95
5		13 '	1.68	110
		14 '	1.04	102
		15 -	0.84	101
		16	1.4	98
		17	0.15	104
0		18	1.77	. 24
		19 -	1.11	107
	3	20	1.73	0
		21 -	0.018	6
		22 ·	0.48	0
5		23 -	1.8	105
		24 .	0.83	94
		25 -	0.78	93
		25	0.78	93

1 unit of polymerase activity = 10 nmoles of total nucleotides incorporated into acid insoluble form in 30 minutes at 75°C. Primed and unprimed colonies were obtained from cells transformed on different days.

TABLE 2

Alterations Relative to pLSM5 (SEQ ID NO: 3)

plasmid	nucleotide position	amino acid position	codon change	amino acid change
p T a r f 2 (SEQ ID NO: 9)	337	73	TTC-CTC	Phe-Leu
pTarf3	193	25	CGC-TGC	Arg-Cys
(SEQ ID NO: 5)	504	128	AAG-AAA	Lys-Lys
p T a r f 5 (SEQ ID NO: 7)	341	74	CGC-CAC	Arg-His

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EXAMPLE 8

Improved Processivity of the Modified Taq Polymerase

Processivity of DNA synthesis by the modified Taq DNA polymerase (REM-T3) was assessed by several trials, with comparison to commercial enzymes and REM-T2. The method using the PCR protocol is novel.

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Trial 1: Gel analysis of processivity by thermal stable DNA polymerases.

M13mp18 template (0.25 pmol/10 μ l) and 5 ³²P-labeled 17-mer (M13/pUC-40, BioLabs) (0.50 pmol/10 μ l) (calculated $t_m = 52^{\circ}$ C) were annealed in 40 μ l of 10 mM Tris-HCl (pH 8.0), and 5 mM MgCl₂. The mixture was incubated for 3 minutes at 90°C, 20 minutes at 42°C, and 15 minutes at room temperature. The reaction mixture was adjusted to 200 μ M each of dNTP, 0.05% Tween 20 and Nonidet P-40, 10 mM Tris-HCl (pH 8.0), 50 mM KCl and 2.5 mM MgCl₂, in a total

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volume of 80 μ l, then incubated at 55°C for 2 minutes without enzyme. Next, 0.94 units of enzyme (AmpliTaqTM (Cetus), Stoffel Fragment(Cetus), REM-T2 or REM-T3)/10 μ l were added to start the reaction. Five μ l aliquiots were removed from the reaction mixture at 0, 15, 30, 45 seconds, and 1, 2, and 5 minutes and added to 5 μ l of stop solution (1 mg/ml each of xylene cyanol and bromphenol blue, 10 mM EDTA in formamide). For gel analysis, 5 μ l were loaded onto a 6% wedge acrylamide/urea gel.

Figure 4 is a schematic depiction of the process and Figure 5 is an autoradiograph showing the results of trial 1.

Trial 2: Gel analysis of processivity by thermal stable DNA polymerases.

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The same method was used as in Trial 1, except 0.22 units of polymerase/10 μ l of reaction mixture were added. In addition, smaller volumes were used for annealing (25 μ l) and reaction mixture (50 μ l).

For trials 1 and 2, the assayed polymerase activity of the AmpliTaqTM was lower than usual. It appears from the gels that the number of actual units of AmpliTaqTM used in the reaction may have been higher that estimated and, therefore, may not be comparable to the other reactions.

Figure 6 shows the results of trial 2. Note that when the amount of polymerase is limiting, REM-T2 (SEQ ID NO: 4) and REM-T3 (SEQ ID NO: 6) have processivities greater than that of the Stoffel fragment.

Trial 3: PCR analysis of processitivity by thermal stable DNA polymerases.

The final volume of PCR reaction was 50 μ l. The buffer contained 67 mM Tris-HCI (pH 8.8), 16 mM (NH₄)₂SO₄, 10 mM beta mercaptoethanol, 2 mM MgCl₂, 6.7 μ M EDTA, and 150 μ M each dNTP. There was an excess of template (0.02 pmol/10 μ l) and primers (each 10 pmol/10 μ l) over enzyme (0.04 units of polymerase/ 10 μ l) for each PCR reaction. The template was pLSM5 (SEQ ID NO: 3), a 5.1 kb plasmid containing Taq DNA polymerase gene and used for sequencing. For the 834-951 primer set, at least 102 nucleotides must be added to the primers to form the 117 base pair product, and for the 1564 -1937 primer set, at least 358 nucleotides must be added to the primer to form the 373 base pair product. The PCR program was 20 sec denaturation at 94°C, 30 sec annealing at 48°C, and 2 min extension at 72°C for 12 cycles.

Figure 7 is a schematic depiction of this process and Figure 8 shows is an autoradiograph showing the results.

Interpretation of Processivity Testing

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Trials I and 2 are based on methodology similar to Innis et al., *Proc. Natl. Acad. Sci.* 85, 9436 (1988); Tabor et al., *J. Biol. Chem.* 262, 16212 (1987); and Wernette et al., *Biochem.* 27, 6046 (1988). The use of a fixed primer for synthesis under conditions of limiting enzyme activity and excess template/primer allows analysis of the length of extension of the primer with minimal chance for re-initiation. Thus, analysis of product size by polyacrylamide/urea gel measures primer extension as a unit event, or processivity of the polymerase (trials 1 and 2).

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Trial 3 is based on a new approach. We reasoned that it would be possible to measure processivity under conditions of PCR. With limiting enzyme concentration and excess primer/template concentration, the probability of re-initiation on a partially extended primer in PCR cycles is very low. Therefore, the length of the observed product (resulting from the complete extension of a primer through the opposing primer) is a measure of processivity. We found that 12 cycles results in sufficient yield to detect products with ethidium bromide on agarose gel. By varying the distance between primers we can determine a processivity range. AmpliTaqTM, REM-T2, and REM-T3 have a processivity of at least 105 nucleotides, but less that 358 nucleotides. Stoffel Fragment, on the other hand has a processivity of less than 105 nucleotides.

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Figure 8 compares the ability of four polymerases to extend a primer 105 nucleotides (Lanes 1-4) or 358 nucleotides (Lanes 5-8) under PCR conditions of excess DNA template (0.02 pmol/10 μ l of reaction) and primer (10 pmol/10 μ l of reaction) and limited polymerase units (0.04 units of polymerase/10 μ l reaction). PCR products are shown on a 3% NuSieve gel. AmpliTaqTM is in lanes 1 and 5, Stoffel Fragment is in lanes 2 and 6, REM-T2 in lanes 3 and 7, and REM-T3 in lanes 4 and 8. Marker lane has ϕ x 174/Hae III.

It is evident from an examination of Figures 6, 7, and 8 that REM-T3 (SEQ ID NO: 6) has a processivity equal to or better than AmpliTaqTM, and much better than the Stoffel fragment. This result demonstrates that the full length polypeptide of the modified *Taq* enzyme confers superior processivity compared to the truncated peptide of the Stoffel enzyme.

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EXAMPLE 9

Misincorporation Rate for Modified Taq DNA Polymerases

Information already published by Barnes, Gene 112, 29-35 (1992) indicates that Taq DNA polymerase which has had the N-terminal region containing the 5' exonuclease domain removed has a diminished misincorporation rate. The information available indicates that such a modified Taq DNA polymerase has a two-fold lower misincorporation rate than native Taq DNA polymerase. Since the evidence presented by Barnes leads to the conclusion that the misincorporation by the Taq DNA polymerase is lowered in the absence of the exonuclease activity, we are motivated to measure the misincorporation rate of the modified Taq DNA polymerases described herein.

The assessment of misincorporation is done by several methodologies:

- 1. The methodology of Barnes uses a specially constructed plasmed with a flanking selectable marker, based on identification of lacZ as an indicator gene. Scoring for misincorporation in the lac gene is by the familiar blue/white test on an indicator dye (XGal). Testing for misincorporation is performed by inserting the plasmids into an indicator bacterial strain following PCR reactions in vitro.
- 2. The methodology of Tindall and Kunkel, Biochemistry 21, 6008-6013 (1988) monitors the fidelity of in vitro DNA synthesis using the lacZ gene for a complementation in a plasmid derived from M13 bacteriophage. Measurement of misincorporation is based on the blue/white test for lacZ function using an indicator dye in the plate. The plasmid derivative contains an open single-stranded gap region

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of 390 nucleotides. This construction allows measurement of the forward mutation rate, or the substantially lower reversion mutation rate for any specific misincorporation constructed. The results found by Kunkel and coworkers, indicate that the native *Taq* DNA polymerase has a base substitution error rate of approximately 1/9000 nucleotides polymerized.

The processivity of our modified *Taq* DNA polymerase is much higher than the processivity of the truncated proteolytic fragment, and since the DNA polymerase literature indicates that misincorporation correlates with re-initiation, our misincorporation rate is considerably improved relative to native *Taq* DNA polymerase.

PCT/US94/13554

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SEQUENCE LISTING

_	(1) GENE	RAL INFORMATION:
5	(i)	APPLICANT: Moses M.D., Robb E.
10	(ii)	TITLE OF INVENTION: Modified Thermo-Resistant DNA Polymerases
10	(iii)	NUMBER OF SEQUENCES: 15
15 20	(iv)	CORRESPONDENCE ADDRESS: (A) ADDRESSEE: Allegretti & Witcoff (B) STREET: 10 South Wacker Drive (C) CITY: Chicago (D) STATE: IL (E) COUNTRY: USA (F) ZIP: 60606
25	(v)	COMPUTER READABLE FORM: (A) MEDIUM TYPE: Floppy disk (B) COMPUTER: IBM PC compatible (C) OPERATING SYSTEM: PC-DOS/MS-DOS (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
30	(vi)	CURRENT APPLICATION DATA: (A) APPLICATION NUMBER: (B) FILING DATE: (C) CLASSIFICATION:
35	(viii)	ATTORNEY/AGENT INFORMATION: (A) NAME: Greenfield Ph.D., Michael S. (B) REGISTRATION NUMBER: 37,142 (C) REFERENCE/DOCKET NUMBER: 93,413
40	(ix)	TELECOMMUNICATION INFORMATION: (A) TELEPHONE: (312)715-1000 (B) TELEFAX: (312)715-1234
	(2) INFO	RMATION FOR SEQ ID NO::1:
45	(i)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 2626 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
50	(ii)	MOLECULE TYPE: cDNA
	(iii)	HYPOTHETICAL: NO
55	(iv)	ANTI-SENSE: NO
	(vi)	ORIGINAL SOURCE: (A) ORGANISM: Thermus aquaticus
60	(ix)	FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1212619
	(ix)	FEATURE:

31

(A) NAME/KEY: mat_peptide (B) LOCATION: 121..2616

(ix) FEATURE:

5

- (A) NAME/KEY: (B) LOCATION: 1..2625
 (D) OTHER INFORMATION: /note= "Native Taq DNA Polymerase nucleotide sequence."

		(xi) SE	QUEN	CE D	ESCR	IPTI	ON:	SEQ	ID N	10::1	L:					
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15	GGG	AGGC	GTT	TCTA	AAAG	cc c	TTCA	GGAC	G CT	ACCC	GGGG	GCG	GGTG	GTG	GAAG	GGTAAC	120
20	ATG Met 1	Arg	GGG Gly	ATG Met	CTG Leu 5	CCC Pro	CTC Leu	TTT Phe	GAG Glu	CCC Pro	Lys	GGC Gly	CGG Arc	GTC Val	CTC Leu 15	CTG Leu	168
20	GTG Val	GAC Asp	GGC	CAC His 20	His	CTG Leu	GCC Ala	TAC Tyr	CGC Arg 25	Thr	TTC Phe	CAC His	GCC Ala	CTG Leu 30	Lys	GGC Gly	216
25	CTC Leu	ACC Thr	ACC Thr 35	AGC Ser	CGG Arg	GGG Gly	GAG Glu	CCG Pro 40	GTG Val	CAG Gln	GCG Ala	GTC Val	TAC Tyr 45	Gly	TTC Phe	GCC Ala	264
30	AAG Lys	AGC Ser 50	Leu	CTC Leu	AAG Lys	GCC Ala	CTC Leu 55	AAG Lys	GAG Glu	GAC Asp	GGG Gly	GAC Asp 60	Ala	GTG Val	ATC	GTG Val	312
35	GTC Val 65	TTT Phe	GAC Asp	GCC Ala	AAG Lys	GCC Ala 70	CCC Pro	TCC Ser	TTC Phe	CGC Arg	CAC His 75	GAG Glu	GCC Ala	TAC Tyr	GCG	GGG Gly 80	360
40	TAC	AAG Lys	GCG Ala	GGC Gly	CGG Arg 85	GCC Ala	CCC Pro	ACG Thr	CCG Pro	GAG Glu 90	Asp	TTT Phe	CCC Pro	CGG Arg	CAA Gln 95	Leu	408
	GCC Ala	CTC Leu	ATC Ile	AAG Lys 100	GAG Glu	CTG Leu	GTG Val	GAC Asp	CTC Leu 105	CTG Leu	GGG Gly	CTG Leu	GCG Ala	CGC Arg 110	Leu	GAG Glu	456
45	GTC Val	CCG Pro	GGC Gly 115	TAC Tyr	GAG Glu	GCG Ala	GAC Asp	GAC Asp 120	GTC Val	CTG Leu	GCC Ala	AGC Ser	CTG Leu 125	GCC Ala	AAG Lys	FÅa FÅG	504
50	GCG Ala	GAA Glu 130	AAG Lys	GAG Glu	GGC Gly	TAC Tyr	GAG Glu 135	GTC Val	CGC Arg	ATC Ile	CTC Leu	ACC Thr 140	GCC Ala	GAC Asp	AAA Lys	GAC Asp	552
55	CTT Leu 145	TAC Tyr	CAG Gln	CTC Leu	CTT Leu	TCC Ser 150	GAC Asp	CGC Arg	ATC Ile	CAC His	GTC Val 155	CTC Leu	CAC His	CCC Pro	GAG Glu	GGG Gly 160	600
60	TAC Tyr	CTC Leu	ATC Ile	ACC Thr	CCG Pro 165	GCC Ala	TGG Trp	CTT Leu	TGG Trp	GAA Glu 170	AAG Lys	TAC Tyr	GGC Gly	CTG Leu	AGG Arg 175	Pro	648
	GAC Asp	CAG Gln	TGG Trp	GCC Ala 180	GAC Asp	TAC Tyr	CGG Arg	GCC Ala	CTG Leu 185	ACC Thr	GGG Gly	GAC Asp	GAG Glu	TCC Ser 190	Asp	AAC Asn	696

								Glu			GCG Ala		Lys	CTG Leu	744
5												Leu		CTG Leu	792
10						Lys								AAG Lys 240	840
15											CTG Leu			GTG Val	888
											AGG Arg			TTT Phe	936
20											GAG Glu			CTG Leu	984
25											CCC Pro 300			GGG Gly	1032
30											CCC Pro			GAT Asp 320	1080
35											GTC Val			CCC Pro	1128
											GCG Ala			CTC Leu	1176
40											GGC Gly			CCG Pro	1224
45	Gly	Asp	Asp	Pro	Met	Leu	Leu	Ala	Tyr	Leu	CTG Leu 380	Asp		AAC Asn	1272
50											GGG Gly				1320
55	 										CTC Leu				1368
											TGG Trp				1416
60											ATG Met				1464

	GT Val	G CG Arg 450	Leu	Asp G GA	C GTO Val	G GCC Ala	TAT Tyr 455	Leu	AGG Arg	GCC Ala	TTC Leu	TCC Ser 460	Leu	GAG Glu	GTG Val	GCC Ala	1512
5	GAG Glu 465	Glu	ATC Ile	GCC Ala	CGC Arg	CTC Leu 470	GAG Glu	GCC Ala	GAG Glu	GTC Val	TTC Phe 475		CTG Leu	GCC Ala	GGC Gly	CAC His 480	1560
10	CCC Pro	TTC Phe	AAC Asn	CTC Leu	AAC Asn 485	TCC Ser	CGG Arg	GAC Asp	CAG Gln	CTG Leu 490	Glu	AGG Arg	GTC Val	CTC Leu	TTT Phe 495	Asp	1608
15	GAG Glu	CTA Leu	GGG Gly	CTT Leu 500	CCC Pro	GCC Ala	ATC Ile	GGC Gly	AAG Lys 505	ACG Thr	GAG Glu	AAG Lys	ACC Thr	GGC Gly 510	Lys	CGC Arg	1656
20	TCC Ser	ACC Thr	AGC Ser 515	GCC Ala	GCC Ala	GTC Val	CTG Leu	GAG Glu 520	GCC Ala	CTC Leu	CGC Arg	GAG Glu	GCC Ala 525	CAC His	CCC Pro	ATC Ile	1704
	GTG Val	GAG Glu 530	AAG Lys	ATC Ile	CTG Leu	CAG Gln	TAC Tyr 535	CGG Arg	GAG Glu	CTC Leu	ACC Thr	AAG Lys 540	CTG Leu	AAG Lys	AGC Ser	ACC Thr	1752
25	TAC Tyr 545	ATT Ile	GAC Asp	CCC Pro	TTG Leu	CCG Pro 550	GAC Asp	CTC Leu	ATC Ile	CAC His	CCC Pro 555	AGG Arg	ACG Thr	GGC Gly	CGC Arg	CTC Leu 560	1800
30	CAC His	ACC Thr	CGC Arg	TTC Phe	AAC Asn 565	CAG Gln	ACG Thr	GCC Ala	ACG Thr	GCC Ala 570	ACG Thr	GGC Gly	AGG Arg	CTA Leu	AGT Ser 575	AGC Ser	1848
35	TCC Ser	GAT Asp	CCC Pro	AAC Asn 580	CTC Leu	CAG Gln	AAC Asn	ATC Ile	CCC Pro 585	GTC Val	CGC Arg	ACC Thr	CCG Pro	CTT Leu 590	GGG Gly	CAG Gln	1896
40	AGG Arg	ATC Ile	CGC Arg 595	CGG Arg	GCC Ala	TTC Phe	ATC Ile	GCC Ala 600	GAG Glu	GAG Glu	GGG Gly	TGG Trp	CTA Leu 605	TTG Leu	GTG Val	GCC Ala	1944
	CTG Leu	GAC Asp 610	TAT Tyr	AGC Ser	CAG Gln	ATA Ile	GAG Glu 615	CTC Leu	AGG Arg	GTG Val	CTG Leu	GCC Ala 620	CAC His	CTC Leu	TCC Ser	GGC Gly	1992
45	GAC Asp 625	Glu	AAC Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	CGG Arg	Asp	Ile	His	Thr	2040
50	GAG Glu	ACC Thr	GCC Ala	AGC Ser	TGG Trp 645	ATG Met	TTC Phe	GGC ·	GTC Val	CCC Pro 650	CGG Arg	GAG Glu	GCC Ala	GTG Val	GAC Asp 655	CCC Pro	2088
55	CTG Leu	ATG Met	CGC Arg	CGG Arg 660	GCG Ala	GCC . Ala	AAG Lys	ACC . Thr	ATC Ile 665	AAC Asn	TTC Phe	GGG Gly	GTC Val	CTC Leu 670	TAC Tyr	GGC Gly	2136
60	ATG Met	TCG Ser	GCC Ala 675	CAC His	CGC Arg	CTC Leu	TCC Ser	CAG Gln 680	GAG Glu	CTA Leu	GCC Ala	ATC Ile	CCT Pro 685	TAC Tyr	GAG Glu	GAG Glu	2184
	GCC Ala	CAG Gln 690	GCC Ala	TTC Phe	ATT Ile	GAG Glu	CGC Arg 695	TAC Tyr	TTT Phe	CAG Gln	AGC Ser	TTC Phe 700	CCC Pro	AAG Lys	GTG Val	CGG Arg	2232

5	GCC Ala 705	Trp	ATT Ile	GAG Glu	AAG Lys	ACC Thr 710	Leu	GAG Glu	GAG Glu	GGC Gly	AGG Arg 715	Arg	CGG Arg	GGG Gly	TAC Tyr	GTG Val 720	2280
	GAG Glu	ACC Thr	CTC Leu	TTC Phe	GGC Gly 725		CGC Arg	CGC Arg	TAC Tyr	GTG Val 730	Pro	GAC Asp	CTA Leu	GAG Glu	GCC Ala 735	Arg	2328
10	GTG Val	AAG Lys	AGC Ser	GTG Val 740	CGG Arg	GAG Glu	GCG Ala	GCC Ala	GAG Glu 745	CGC Arg	ATG Met	GCC Ala	TTC Phe	AAC Asn 750	Met	CCC Pro	2376
15	GTC Val	CAG Gln	GGC Gly 755	ACC Thr	GCC Ala	GCC Ala	GAC Asp	CTC Leu 760	ATG Met	AAG Lys	CTG Leu	GCT Ala	ATG Met 765	GTG Val	AAG Lys	CTC Leu	2424
20	TTC Phe	CCC Pro 770	AGG Arg	CTG Leu	GAG Glu	GAA Glu	ATG Met 775	GGG Gly	GCC Ala	AGG Arg	ATG Met	CTC Leu 780	CTT Leu	CAG Gln	GTC Val	CAC His	2472
25	GAC Asp 785	GAG Glu	CTG Leu	GTC Val	CTC Leu	GAG Glu 790	GCC Ala	CCA Pro	AAA Lys	GAG Glu	AGG Arg 795	GCG Ala	GAG Glu	GCC Ala	GTG Val	GCC Ala 800	2520
···	C GG A rg	CTG Leu	GCC Ala	AAG Lys	GAG Glu 805	GTC Val	ATG Met	GAG Glu	GGG Gly	GTG Val 810	TAT Tyr	CCC Pro	CTG Leu	GCC Ala	GTG Val 815	CCC Pro	2568
30	CTG Leu	GAG Glu	GTG Val	GAG Glu 820	GTG Val	GGG Gly	ATA Ile	GGG Gly	GAG Glu 825	GAC Asp	TGG Trp	CTC Leu	TCC Ser	GCC Ala 830	AAG Lys	GAG Glu	2616
35	TGAT	TACC	ACC														2626
	(2)		_			SEQ CHAF											
40		'	(1)	(A)	LEN TYP	GTH: PE: a POLOG	832 umino	ami aci	ino a id		3						
45		•				TYPE	_) ID	NO::	2:					
	Met 1	Arg	Gly	Met	Leu 5	Pro	Leu	Phe	Glu	Pro 10	Lys	Gly	Arg	Val	Leu 15	Leu	
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50	Val	Phe	Asp	Ala	Lys	Ala	Pro	Ser	Phe	Arg	His	Glu	Ala	Tyr	Gly	Gly	

	Туг	Lye	Ala	Gly	Arg 85	Ala	Pro	Thr	Pro	90		Phe	Pro	Arg	Gln 95	
5	Ala	Leu	Ile	Lys 100	Glu	Leu	Val	Asp	Leu 105	Leu	Gly	Leu	Ala	Arg	Leu	Glu
	Val	. Pro	Gly 115	Tyr	Glu	Ala	Yab	120	Val	Leu	Ala	Ser	Leu 125		Lys	Lys
10	Ala	Glu 130	Lys	Glu	Gly	Tyr	Glu 135	Val	Arg	Ile	Leu	Thr 140	Ala	Asp	Lys	Asp
15	Leu 145	Tyr	Gln	Leu	Leu	Ser 150	Asp	Arg	Ile	His	Val 155	Leu	His	Pro	Glu	Gly 160
	Tyr	Leu	Ile	Thr	Pro 165	Ala	Trp	Leu	Trp	Glu 170	Lys	Tyr	Gly	Leu	Arg 175	Pro
20	Asp	Gln	Trp	Ala 180	Asp	Tyr	Arg	Ala	Leu 185	Thr	Gly	Asp	Glu	Ser 190	Asp	Asn
	Leu	Pro	Gly 19	Val 5	Lys	Gly	Ile	Gly 20		Lys	Thr	Ala	Arg 20		Leu	Leu
25	Glu	Glu 210	Trp	Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	Asn 220	Leu	Asp	Arg	Leu
30	Lys 225	Pro	Ala	Ile	Arg	Glu 230	Lys	Ile	Leu	Ala	His 235	Met	Asp	Asp	Leu	Lys 240
	Leu	Ser	Trp	Asp	Leu 245	Ala	Lys	Val	Arg	Thr 250	Asp	Leu	Pro	Leu	Glu 255	Val
35				260					265					270	Ala	
			275					280					285		Leu	
40		290					295					300			Glu	_
45	305					310					315				Ala	320
					325					330					Ala 335	
50				340					345					350	Leu	
			355					360					365		Leu	
55		370					375					380			Ser	
60	385					390					395					400
	Glu	Ala	Gly	Glu	Arg 405	Ala	Ala	Leu		Glu 410	Arg	Leu	Phe	Ala	Asn 415	Leu

	Trp	Gly	Arg	Leu 420	Glu	Gly	Glu	Glu	Arg 425	Leu	Leu	Trp	Leu	Tyr 430	Arg	Glu
5	Val	Glu	Arg 435	Pro	Leu	Ser	Ala	Val 440	Leu	Ala	His	Met	Glu 445	Ala	Thr	Gly
	Val	Arg 450	Leu	yab	Val	Ala	Tyr 455	Leu	Arg	Ala	Leu	Ser 460	Leu	Glu	val	Ala
10	Glu 465	Glu	Ile	Ala	Arg	Leu 470		Ala	Glu	Val	Phe 475	Arg	Leu	Ala	Gly	His 480
15	Pro	Phe	Asn	Leu	Asn 485	Ser	Arg	Asp	Gln	Leu 490	Glu	Arg	Val	Leu	Phe 495	Asp
13	Glu	Leu	Gly	Leu 500	Pro	Ala	Ile	Gly	Lys 505	Thr	Glu	Lys	Thr	Gly 510	Lys	Arg
20	Ser	Thr	Ser 515	Ala	Ala	Val	Leu	Glu 520	Ala	Leu	Arg	Glu	Ala 525	His	Pro	Ile
	Val	Glu 530	Lys	Ile	Leu	Gln	Tyr 535	Arg	Glu	Leu	Thr	Lys 540	Leu	Lys	Ser	Thr
25	Tyr 545	Ile	Asp	Pro	Leu	Pro 550	Asp	Leu	Ile	His	Pro 555	Arg	Thr	Gly	Arg	Leu 560
30	His	Thr	Arg	Phe	Asn 565	Gln	Thr	Ala	Thr	Ala 570	Thr	Gly	Arg	Leu	Ser 575	Ser
30	Ser	Asp	Pro	Asn 580	Leu	Gln	Asn	Ile	Pro 585	Val	Arg	Thr	Pro	Leu 590	Gly	Gln
35	Arg	Ile	Arg 595	Arg	Ala	Phe	Ile	Ala 600	Glu	Glu	Gly	Trp	Leu 605	Leu	Val	Ala
	Leu	Asp 610	Tyr	Ser	Gln	Ile	Glu 615	Leu	Arg	Val	Leu	Ala 620	His	Leu	Ser	Gly
40	Asp 625	Glu	Asn	Leu	Ile	Arg 630	Val	Phe	Gln	Glu	Gly 635	Arg	Asp	Ile	His	Thr 640
45		Thr			645					650					655	
10		Met		660					665					670		
50	Met	Ser	Ala 675		Arg	Leu	Ser	Gln 680	Glu	Leu	Ala	Ile	Pro 685	Tyr	Glu	Glu
		690					695					700				Arg
55	705	-			_	710					715					Val 720
60	Glu	Thr	Leu	Phe	Gly 725	Arg	Arg	Arg	Tyr	Val 730	Pro	Asp	Leu	Glu	Ala 735	Arg
30	Val	Lys	Ser	Val 740	Arg	Glu	Ala	Ala	Glu 745	Arg	Met	Ala	Phe	Asn 750	Met	Pro

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	Val	Gln	Gly 755	Thr	Ala	Ala	Asp	Leu 760	Met	Lys	Leu	Ala	Met 765	Val	Lys	Leu	
5	Phe	Pro 770	Arg	Leu	Glu	Glu	Met 775	Gly	Ala	Arg	Met	Leu 780	Leu	Gln	Val	His	
	Asp 785	Glu	Leu	Val	Leu	Glu 790	Ala	Pro	Lys	Glu	Arg 795	Ala	Glu	Ala	Val	Ala 800	
10	Arg	Leu	Ala	Lys	Glu 805	Val	Met	Glu	Gly	Val 810	Tyr	Pro	Leu	Ala	Val 815	Pro	
15	Leu	Glu	Val	Glu 820	Val	Gly	Ile	Gly	Glu 825	Asp	Trp	Leu	Ser	Ala 830	Lys	Glu	
	(2)	INFO	RMAI	NOI	FOR	SEQ	ID N	10::3	3:								
20		(i)	(E (C	() LE () TY () SI	NGTH PE: RAND	IARAC I: 26 nucl EDNE GY:	26 b eic SS:	ase acid sing	pair l	's							
25		(ii)	MOL	ECUL	E TY	PE:	DNA	(gen	omic	:)							
	((iii)	HYP	OTHE	TICA	L: N	0										
30		(iv)	TNA	I-SE	NSE:	NO											
		(vi)				URCE SM:		mus	aqua	ticu	s						
35		(ix)	(B) NA	ME/K CATI HER nuc	leot	repl RMAT ide	ace(ION: alte	89, /no rati	te= on at	"Thi	tion	89	of t	he na	ts in a ative Taq	DNA
40		/iv\	FEA	ממווים		ymer	ase	nucı	eoti	ae s	eque	nce	or C	to	G."		
45		(11)	(A (B) NA) LO	ME/K CATI HER nuc DNA	EY: ON: INFO leot pol ults	repl RMAT ide ymer	ace(ION: alte ase	934, /no rati nucl	te= on a eotic	"Thi t po de s	siti eque:	on 9 nce	34 o of T	f th	ts in a e native A. This le."	Taq
50		(ix)	(A (B) NA	ME/K CATION HER :	leot	repl RMAT ide	ace(' ION: alte:	962, /no rati	te= '	Thi:	siti	on 9	62 o	f the	ts in a e native C. This	Taq
55					res	ults	in	an ai	mino	acio	d cha	ange	of :	Leu	to P	ro."	
60		(ix)	(A (B) NAI	ME/KI CATIO HER : nuc: DNA	EY: none in the second	repla RMAT: ide a ymera	ace() ION: alter ase :	not) ratio nucle	te= ' on at eotic	This pos de se	itic	n 25	35 c	f th	ts in a e native A. This	Taq

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	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1212619	
5	(ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 1212616	
10	<pre>(ix) FEATURE: (A) NAME/KEY: - (B) LOCATION: 12619 (D) OTHER INFORMATION: /note= "pLSM5"</pre>	
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::3:	
	AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA	60
20	GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC	120
20	ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15	168
25	GTG GAC GGC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly 25 30	216
30	CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45	264
35	AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG Lys Ser Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val 50 55 60	312
40	GTC TTT GAC GCC AAG GCC CCC TCC TTC CGC CAC GAG GCC TAC GGG GGG Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly 65 70 75 80	360
40	TAC AAG GCG GGC CGG GCC CCC ACG CCG GAG GA	408
45	GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu 100 105 110	456
50	GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAG Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys 115 120 125	504
55	GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp 130 135 140	552
60	CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly 145 150 155 160	003
	TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AAG TAC GGC CTG AGG CCC Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro 165 170 175	648

	GA As	C CA p Gl	G TG n Tr	G GCC P Ala 180	a vai	TAC Tyr	C CGC	G GCC g Ala	C CTC a Let 189	ı Th:	GGG Gly	GAC	GAC P Gl	TCC u Se 19	r As	C AAC p Asn	696
5	Dei	4 F.L	19	y va . 5	LLYE	. GIŽ	7 116	200) GIG	ı Lyı	3 Thi	r Ala	20:	g Ly	a, Le	r CTG u Leu	744
10	GI	21	0	b GTZ	ser	Leu	215	Ala	Let	l Let	ı Lye	220	ı Let	ı Yel	p Ar	CTG g Leu	792
15	225	FI) AI	a 116	e Arg	230	rys	, lle	. Leu	Ala	His 235	Met	Asr	As _I	Le	AAG Lys 240	840
20	Deu	s sei	LIL		245	ALA	гля	vai	Arg	250	Asp	Leu	Pro	Leu	255	Val	888
25	Asp	Pile	: MIG	260	Arg	Arg	GIU	Pro	265	Arg	Glu	Arg	Leu	Arg 270	Ala	Ile	936
25	Leu	GIU	275		. GIU	Pne	GIĀ	280	Pro	Leu	His	Glu	Phe 285	Gly	Leu	Leu	984
30	Giu	290	PIO		ATA	Leu	295	GIU	Ala	Pro	Trp	Pro 300	Pro	Pro	Glu	Gly	1032
35	305	Pne	Val	GGC	Pne	310	Leu	Ser	Arg	Lys	Glu 315	Pro	Met	Trp	Ala	Asp 320	1080
40	Leu	reu	AIG	CTG Leu	325	Ala	Ala	Arg	Gly	330	Arg	Val	His	Arg	Ala 335	Pro	1128
45	GIU	PIO	lyr	AAA Lys 340	Ala	ren	Arg	Asp	145 345	Lys	Glu	Ala	Arg	Gly 350	Leu	Leu	1176
43	VIG	гув	355	CTG Leu	ser	vai	Leu	360	Leu	Arg	Glu	Gly	Leu 365	Gly	Leu	Pro	1224
50	PIO	370	АБР	GAC Asp	Pro .	Met .	1 eu 375	Leu	Ala	Tyr	Leu	Leu 380	Asp	Pro	Ser	Asn	1272
55	385	THE	PIO	GAG Glu	GIY :	390	AIA .	Arg .	Arg	Tyr	Gly 395	Gly (Glu	Trp	Thr	Glu 400	1320
60	GIU	NIG	GIY		405	Ala A	Ala :	Leu :	Ser (Glu . 410	Arg :	Leu :	Phe	Ala	Asn 415	Leu	1368
	TGG (GGG	ALG	CTT (Leu (420	GAG G Glu G	GG G	GAG (Glu A	AGG C Arg 1 425	CTC (Leu :	CTT 1 Leu :	Trp 1	Leu	TAC (Tyr 430	CGG (Arg	GAG Glu	1416

	GTG Val	GAG Glu	AGG Arg 435	CCC Pro	CTT Leu	TCC Ser	GCT Ala	GTC Val 440	CTG Leu	GCC Ala	CAC His	ATG Met	GAG Glu 445	GCC Ala	ACG Thr	GGG Gly	1464
5	GTG Val	CGC Arg 450	CTG Leu	GAC Asp	GTG Val	GCC Ala	TAT Tyr 455	CTC Leu	AGG Arg	GCC Ala	TTG Leu	TCC Ser 460	CTG Leu	GAG Glu	GTG Yal	GCC Ala	1512
10	GAG Glu 465	Glu	Ile	Ala	Arg	Leu 470	Glu	Ala	Glu	Val	Pne 475	Arg	Leu	Ala	GIÀ	480	1560
15	CCC Pro	TTC Phe	AAC Asn	CTC Leu	AAC Asn 485	TCC Ser	CGG Arg	GAC Asp	CAG Gln	CTG Leu 490	GAA Glu	AGG Arg	GTC Val	CTC Leu	TTT Phe 495	Asp	1608
20	GAG Glu	CTA Leu	GGG Gly	CTT Leu 500	CCC Pro	GCC Ala	ATC Ile	GGC Gly	AAG Lys 505	ACG Thr	GAG Glu	AAG Lys	ACC Thr	GGC Gly 510	Lys	CGC Arg	1656
20	TCC Ser	ACC Thr	AGC Ser 515	GCC Ala	GCC Ala	GTC Val	CTG Leu	GAG Glu 520	GCC Ala	CTC Leu	CGC Arg	GAG Glu	GCC Ala 525	His	CCC Pro	ATC Ile	1704
25	GTG Val	GAG Glu 530	Lys	ATC Ile	CTG Leu	CAG Gln	TAC Tyr 535	CGG Arg	GAG Glu	CTC Leu	ACC Thr	AAG Lys 540	CTG Leu	AAG Lys	AGC Ser	ACC Thr	1752
30 .	TAC Tyr 545	ATT Ile	GAC Asp	CCC Pro	TTG Leu	CCG Pro 550	GAC Asp	CTC Leu	ATC Ile	CAC His	CCC Pro 555	Arg	ACG Thr	GGC	CGC Arg	CTC Leu 560	1800
35	CAC His	ACC Thr	CGC Arg	TTC Phe	AAC Asn 565	CAG Gln	ACG Thr	GCC Ala	ACG Thr	GCC Ala 570	Thr	GGC Gly	AGG Arg	CTA Leu	AGT Ser 575	ser	1848
40	TCC Ser	GAT Asp	CCC Pro	AAC Asn 580	Leu	CAG Gln	AAC Asn	ATC Ile	CCC Pro 585	Val	CGC Arg	ACC Thr	CCG	CTT Leu 590	GTA	CAG Gln	1896
40	AGG Arg	ATC Ile	CGC Arg 595	Arg	GCC Ala	TTC Phe	ATC Ile	GCC Ala 600	Glu	GAG Glu	GGG Gly	TGG	CTA Leu 605	Leu	GTG Val	GCC Ala	1944
45	Leu	Asp	Tyr	Ser	Gln	Ile	Glu	CTC Leu	Arg	Val	CTG Leu	GCC Ala 620	. Hie	CTC Leu	TCC Ser	GGC Gly	1992
50	GAC Asp 625	GAG Glu	AAC Asn	CTG Leu	ATC Ile	CGG Arg 630	Val	TTC Phe	CAG Gln	GAG Glu	GGG Gly 635	Arg	GAC Asi	ATC 11e	CAC His	ACG Thr 640	2040
55	GAG Glu	ACC Thr	GCC	AGC Ser	TGG Trp 645	Met	TTC Phe	GGC Gly	GTC Val	CCC Pro 650	Arg	GAG J Glu	GCC Ala	GTG Val	GAC Asj 659	CCC P Pro	2088
	CTG Leu	ATG Met	CGC Arg	CGG Arg	Ala	GCC Ala	AAG Lys	ACC Thr	ATC Ile 665	Asn	TTC Phe	GGG Gly	GTC Val	CTC Leu 670	ı Tyı	GGC Gly	2136
60	ATG Met	TCG Ser	GCC Ala 675	Hie	CGC Arg	CTC Leu	TCC Ser	CAG Glr 680	Glu	CTA Leu	GCC Ala	ATC A Ile	CCT Pro 68!	о Туз	GAG Glu	GAG u Glu	2184

	GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC TTC CCC AAG GTG CGG Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg 690 695 700	2232
5	GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val 705 710 715 720	2280
10	GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG Glu Thr Leu Phe Gly Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg 725 730 735	2328
15	GTG AAG AGC GTG CGG GAG GCC GAG CGC ATG GCC TTC AAC ATG CCC Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro 740 745 750	2376
- 20	GTC CAG GGC ACC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu 755 760 765	2424
	TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His 770 780	2472
25	GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala 785 790 795 800	2520
30	CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro 805 810 815	2568
35	CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu 820 825 830	2616
	TGATACCACC	2626
40	(2) INFORMATION FOR SEQ ID NO::4:	
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	(ii) MOLECULE TYPE: protein	
50	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::4:	
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	Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45	
60	Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val	

	Val 65	Phe	Asp	Ala	Lys	Ala 70	Pro	Ser	Phe	Arg	His 75	Glu	Ala	Tyr	Gly	Gly 80
5	Tyr	Lys	Ala	Gly	Arg 85	Ala	Pro	Thr	Pro	Glu 90	Asp	Phe	Pro	Arg	Gln 95	Leu
	Ala	Leu	Ile	Lys 100	Glu	Leu	Val	Asp	Leu 105	Leu	Gly	Leu	Ala	A rg 110	Leu	Glu
10	Val	Pro	Gly 115	Tyr	Glu	Ala	Asp	Asp 120	Val	Leu	Ala	Ser	Leu 125	Ala	Lys	Lys
15	Ala	Glu 130	Lys	Glu	Gly	Tyr	Glu 135	Val	Arg	Ile	Leu	Thr 140	Ala	Asp	Lys	Asp
1.5	Leu 145	Tyr	Gln	Leu	Leu	Ser 150	Asp	Arg	Ile	His	Val 155	Leu	His	Pro	Glu	Gly 160
20	Tyr	Leu	Ile	Thr	Pro 165	Ala	Trp	Leu	Trp	Glu 170	Lys	Tyr	Gly	Leu	Arg 175	Pro
	Asp	Gln	Trp	Ala 180	Asp	Tyr	Arg	Ala	Leu 185	Thr	Gly	yab	Glu	Ser 190	yab	Asn
25	Leu	Pro	Gly 195	Val	Lys	Gly	Ile	Gly 200	Glu	Lys	Thr	Ala	Arg 205	Lys	Leu	Leu
30	Glu	Glu 210	Trp	Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	Asn 220	Leu	Asp	Arg	Leu
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35	Leu	Ser	Trp	Asp	Leu 245	Ala	Lys	Val	Arg	Thr 250	Asp	Leu	Pro	Leu	Glu 255	Val
	_			260					265				Leu	270	•	
40			275					280					Phe 285			
45		290					295					300	Pro			
	305					310					315		Met			320
50					325					330			His		335	
				340					345				Arg	350		-
55		_	355					360					Leu 365			
60		370					375					380				
J.	Thr 385		Pro	Glu	Gly	Val 390	Ala	Arg	Arg	Tyr	Gly 395	Gly	Glu	Trp	Thr	Gl:

	Gl	u Ala	a Gly	y Glu	405	Ala	a Ala	a Le	ı Se	r Glu 410	ı Arç	J Let	ı Phe	e Ala	41:	Leu
5	Tr	Gl:	y Arg	2 Let 420	ı Glu	Gly	/ Glu	Glu	425	g Leu	ı Lev	Tr	Lev	1 Ty:		g Glu
	Vá	al G	lu Ar 435	g Pr	o Le	u Se	er Al	a Va	al Le	eu Al	a Hi	.в Ме	et G] 445	Lu Al	a Ti	r Gly
10	Val	Arg 450	J Leu	a Asp	Val	Ala	Tyr 455	Leu	Arç	, Ala	Leu	Ser 460		Glu	Val	Ala
15	Glu 465	Glu	ılle	Ala	Arg	Leu 470	Glu	Ala	Glu	Val	Phe 475		Leu	Ala	Gly	His 480
	Pro	Phe	e Asn	Leu	Asn 485	Ser	Arg	Asp	Gln	Leu 490	Glu	Arg	Val	Leu	Phe 495	Asp
20	Glu	Leu	Gly	Leu 500	Pro	Ala	Ile	Gly	Lys 505	Thr	Glu	Lys	Thr	Gly 510		Arg
	Ser	Thr	Ser 515	Ala	Ala	Val	Leu	Glu 520	Ala	Leu	Arg	Glu	Ala 525		Pro	Ile
25	Val	Glu 530	Lys	Ile	Leu	Gln	Tyr 535	Arg	Glu	Leu	Thr	Lys 540	Leu	Lys	Ser	Thr
30	Tyr 545	Ile	Asp	Pro	Leu	Pro 550	yab	Leu	Ile	His	Pro 555	Arg	Thr	Gly	Arg	Leu 560
	His	Thr	Arg	Phe	Asn 565	Gln	Thr	Ala	Thr	Ala 570	Thr	Gly	Arg	Leu	Ser 575	Ser
35	Ser	Asp	Pro	Asn 580	Leu	Gln	Asn	Ile	Pro 585	Val	Arg	Thr	Pro	Leu 590	Gly	Gln
	Arg	Ile	Arg 595	Arg	Ala	Phe	Ile	Ala 600	Glu	Glu	Gly	Trp	Leu 605	Leu	Val	Ala
40	Leu	Asp 610	Tyr	Ser	Gln	Ile	Glu 615	Leu	Arg	Val	Leu	Ala 620	His	Leu	Ser	Gly
45	Asp 625	Glu	Asn	Leu	Ile	Arg 630	Val	Phe	Gln	Glu	Gly 635	Arg	Asp	Ile	His	Thr 640
	Glu	Thr	Ala	Ser	Trp 645	Met	Phe	Gly	Val	Pro 650	Arg	Glu	Ala	Val	Asp 655	Pro
50	Leu	Met	Arg	Arg 660	Ala	Ala	Lys	Thr	11e 665	Asn	Phe	Gly	Val	Leu 670	Tyr	Gly
	Met	Ser	Ala 675	His	Arg	Leu	Ser	Gln 680	Glu	Leu	Ala	Ile	Pro 685	Tÿr	Glu'	Glu
55	Ala	Gln 690	Ala	Phe	Ile	Glu	Arg 695	Tyr	Phe	Gln	Ser	Phe 700	Pro	Lys	Val	Arg
60	Ala 705	Trp	Ile	Glu	Lys	Thr 710	Leu	Glu	Glu	Gly	Arg 715	Arg	Arg	Gly	-	Val 720
	Glu	Thr	Leu	Phe	Gly . 725	Arg	Arg	Arg	Tyr	Val 730	Pro .	Asp	Leu	Glu	Ala 735	

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	Val	Lув	Ser	Val 740	Arg	Glu	Ala	Ala	Glu 745	Arg	Met	Ala	Phe	Asn 750		Pro	
5	Val	Gln	Gly 755	Thr	Ala	Ala	Asp	Leu 760	Met	Lys	Leu	Ala	Met 765	Val	rys.	Leu	
	Phe	Pro 770	Arg	Leu	Glu	Glu	Met 775	Gly	Ala	Arg	Met	Leu 780	Leu	Gln	Val	His	
10	А вр 785	Glu	Leu	Val	Leu	Glu 790	Ala	Pro	Lys	Glu	Arg 795	Ala	Glu	Ala	Val	Ala 800	
15	Arg	Leu	Ala	Lys	Glu 805	Val	Met	Glu	Gly	Val 810	Tyr	Pro	Leu	Ala	Val 815	Pro	
	Leu	Glu	Val	Glu 820	Val	Gly	Ile	Gly	Glu 825	Asp	Trp	Leu	Ser	Ala 830		Glu	
20	(2)	INFO															
25		(i)	(<i>I</i> (E	() LE () TY () SI	NGTH PE: RAND	nucl	26 b eic	STIC ase acid sing	pair I	s							•
- 0								(gen	omic	;)							
30	((iii) (iv)		OTHE			10										
35		(vi)				URCE SM:		mus	aqua	ticu	s				4		
10		(ix)	(A (E) LO	ME/K CATI HER nuc	ON: INFO leot	repl RMAT ide	ION: alte	89, /no rati	"g") te= on at de s	"Thi pos	tior	89	of t	he na	ts in a ative Ta	ı aq DNA
15		(ix)	(A (B) LO	ME/K CATI HER nuc	ON: INFO leot	repl RMAT ide	ION: alte	934, /no rati	on a	"Thi t po	siti	on 9	34 o	f th	ts in a e nativ	e Taq
50															to I		.s
55		(ix)	(A (B) NA	ME/K CATI HER nuc DNA	ON: INFO leot pol	repl RMAT ide ymer	ION: alte ase	962, /no rationuclo	on a eoti	"This t pos de s	siti eque:	on 9 nce (62 o of T	esul f the to P		e Taq
50		(ix)	(A (B) NA	ME/KI CATIO	ON:	repla	tion ace(; ION:	2535	, "a' te= '	") "This	s mut	catio	on r	esul	ts in a	

45

nucleotide alteration at position 2535 of the native Taq DNA polymerase nucleotide sequence of G to A. This mutation is conservative."

5		(ix	(A) N B) L	AME/ OCAT THER	ION:	rep ORMA	lace TION	:(193 :: /n	ote=	"Th	nis n	nutat	ion	char	nges th	e
10					po	lyme	rase	fro Cys.	m C 1	to T	res	sulti	ing i	n an	ami	no acid	d change
15		(ix	C	A) N B) L	AME/ OCAT THER nu	ION: INF cleo	rep ORMA tide	lace TION at	(504 : /n posi	ote= tion	"Th	of	the	nati	ve T	ges th ag DNA ve in n	e ature."
20		(ix) FE. (A) N.	e: ame/ ocat	KEY: ION:	CDS 121	26	19								
25		(ix		A) N.					tide 16								
30		(ix	(1	(A) : B) L	NAME OCAT	ION:	1		: /n	ote=	"pT	arf3	•				
35		-	-						SEQ				~~~~				
																GGAGA GGTAAC	60 120
40															CTC Leu 15		168
45 .															AAG Lys		216
50															TTC Phe		264
5.5													Ala		ATC Ile		312
															GGG Gly		360
50	TAC Tyr	AAG Lys	GCG Ala	GGC Gly	CGG Arg 85	GCC Ala	CCC Pro	ACG Thr	CCG Pro	GAG Glu 90	yeb GyC	TTT Phe	CCC Pro	cgg Arg	CAA Gln 95	CTC Leu	408

	GCC Ala	CTC Leu	ATC Ile	AAG Lys 100	GAG Glu	CTG Leu	GTG Val	GAC Asp	CTC Leu 105	Leu	GGG Gly	CTG Leu	GCG Ala	CGC Arg	Leu	GAG Glu	456
5	GTC Val	CCG Pro	GGC Gly 115	TAC Tyr	GAG Glu	GCG Ala	GAC Asp	GAC Asp 120	Val	CTG Leu	GCC Ala	AGC Ser	CTG Leu 125	Ala	AAG Lys	AAA Lys	504
10	GCG Ala	GAA Glu 130	Lys	GAG Glu	GGC Gly	TAC Tyr	GAG Glu 135	Val	CGC Arg	ATC	CTC Leu	ACC Thr 140	Ala	GAC Asp	AAA Lys	GAC Asp	552
15	CTT Leu 145	TAC Tyr	CAG Gln	CTC Leu	CTT Leu	TCC Ser 150	GAC Asp	CGC	ATC Ile	CAC His	GTC Val 155		CAC His	CCC Pro	GAG Glu	GGG Gly 160	600
20	TAC Tyr	CTC Leu	ATC Ile	ACC Thr	CCG Pro 165	GCC Ala	TGG Trp	CTT Leu	TGG Trp	GAA Glu 170	AAG Lys	TAC Tyr	GGC	CTG Leu	AGG Arg 175	Pro	648
20	GAC Asp	CAG Gln	TGG Trp	GCC Ala 180	GAC Asp	TAC Tyr	CGG Arg	GCC Ala	CTG Leu 185	ACC Thr	GGG Gly	GAC Asp	GAG Glu	TCC Ser 190	Asp	AAC Asn	696
25	CTT Leu	CCC Pro	GGG Gly 195	GTC Val	AAG Lys	GGC Gly	ATC Ile	GGG Gly 200	GAG Glu	AAG Lys	ACG Thr	GCG Ala	AGG Arg 205	AAG Lys	CTT Leu	CTG Leu	744
30	GAG Glu	GAG Glu 210	TGG Trp	GGG	AGC Ser	CTG Leu	GAA Glu 215	GCC Ala	CTC Leu	CTC Leu	AAG Lys	AAC Asn 220	CTG Leu	GAC Asp	CGG Arg	CTG Leu	792
35	AAG Lys 225	CCC Pro	GCC Ala	ATC Ile	CGG Arg	GAG Glu 230	AAG Lys	ATC Ile	CTG Leu	GCC Ala	CAC His 235	ATG Met	GAC Asp	GAT Asp	CTG Leu	AAG Lys 240	840
40												CTG Leu					888
40	GAC Asp	TTC Phe	GCC Ala	AAA Lys 260	AGG Arg	CGG Arg	GAG Glu	CCC Pro	GAC Asp 265	CGG Arg	GAG Glu	AGG Arg	CTT Leu	AGG Arg 270	GCC Ala	ATT Ile	936
45	CTG Leu	GAG Glu	Arg	CTT Leu	Glu	Phe	GGC Gly	AGC Ser 280	CCC Pro	CTC Leu	CAC His	GAG Glu	TTC Phe 285	GGC Gly	CTT Leu	CTG Leu	984
50												CCC Pro 300					1032
55	GCC Ala 305	TTC Phe	GTG Val	GGC Gly	TTT Phe	GTG Val 310	CTT Leu	TCC Ser	CGC Arg	AAG Lys	GAG Glu 315	CCC Pro	ATG Met	TGG Trp	GCC Ala	GAT Asp 320	1080
60												GTC Val					1128
60												GCG Ala					1176

									-							
	GCC AA Ala Ly	A GAC B Asp 355	Leu	AGC Ser	GTT Val	CTG Leu	GCC Ala 360	Leu	AGG Arg	GAA Glu	GGC Gly	CTT Leu 365	Gly	CTC Leu	CCG Pro	1224
5	CCC GG Pro Gl	qaA y	GAC Asp	CCC Pro	ATG Met	CTC Leu 375	CTC Leu	GCC Ala	TAC Tyr	CTC Leu	CTG Leu 380	Asp	CCT Pro	TCC Ser	AAC Asn	1272
10	ACC AC Thr Th 385	C CCC r Pro	GAG Glu	GGG Gly	GTG Val 390	GCC Ala	CGG Arg	CGC Arg	TAC Tyr	GGC Gly 395	GGG Gly	GAG Glu	TGG Trp	ACG Thr	GAG Glu 400	1320
15	GAG GC Glu Al	G GGG G Gly	GAG Glu	CGG Arg 405	GCC Ala	GCC Ala	CTT	TCC Ser	GAG Glu 410	AGG Arg	CTC Leu	TTC Phe	GCC Ala	AAC Asn 415	Leu	1368
20	TGG GGG Trp Gl	AGG Arg	CTT Leu 420	GAG Glu	GGG Gly	GAG Glu	GAG Glu	AGG Arg 425	CTC Leu	CTT Leu	TGG Trp	CTT Leu	TAC Tyr 430	CGG Arg	GAG Glu	1416
20	GTG GA	AGG Arg 435	CCC Pro	CTT Leu	TCC Ser	GCT Ala	GTC Val 440	CTG Leu	GCC Ala	CAC His	ATG Met	GAG Glu 445	GCC Ala	ACG Thr	GGG Gly	1464
25	GTG CGG Val Arc 45	g Leu	GAC Asp	GTG Val	GCC Ala	TAT Tyr 455	CTC Leu	AGG Arg	GCC Ala	TTG Leu	TCC Ser 460	CTG Leu	GAG Glu	GTG Val	GCC Ala	1512
30	GAG GAG Glu Glu 465	ATC 1 lle	GCC Ala	CGC Arg	CTC Leu 470	GAG Glu	GCC Ala	GAG Glu	GTC Val	TTC Phe 475	CGC Arg	CTG Leu	GCC Ala	GGC Gly	CAC His 480	1560
35	CCC TTO	AAC Asn	CTC Leu	AAC Asn 485	TCC Ser	CGG Arg	GAC Asp	CAG Gln	CTG Leu 490	GAA Glu	AGG Arg	GTC Val	CTC Leu	TTT Phe 495	Asp Asp	1608
· 40	GAG CT	A GGG	CTT Leu 500	CCC Pro	GCC Ala	ATC Ile	GGC Gly	AAG Lys 505	ACG Thr	GAG Glu	AAG Lys	ACC Thr	GGC Gly 510	AAG Lys	CGC Arg	1656
40	TCC ACC	AGC Ser 515	GCC Ala	GCC Ala	GTC Val	CTG Leu	GAG Glu 520	GCC Ala	CTC Leu	CGC Arg	GAG Glu	GCC Ala 525	CAC His	CCC Pro	ATC Ile	1704
45	GTG GAG Val Glo 53	ı Lys	ATC Ile	Leu	Gln	Tvr	Ara	Glu	Leu	Thr	Lvs	CTG Leu	Lvs	AGC Ser	ACC Thr	1752
50	TAC ATT	r GAC	CCC Pro	TTG Leu	CCG Pro 550	GAC Asp	CTC Leu	ATC Ile	CAC His	CCC Pro 555	AGG Arg	ACG Thr	GJA GGC	Arg	CTC Leu 560	1800
55	CAC AC	c CGC r Arg	TTC Phe	AAC Asn 565	CAG Gln	ACG Thr	GCC Ala	ACG Thr	GCC Ala 570	ACG Thr	GGC Gly	AGG Arg	CTA Leu	AGT Ser 575	AGC Ser	1848
60	TCC GA	r ccc Pro	AAC Asn 580	CTC Leu	CAG Gln	AAC Asn	ATC Ile	CCC Pro 585	GTC Val	CGC Arg	ACC Thr	CCG Pro	CTT Leu 590	GGG Gly	CAG Gln	1896
60	AGG AT	C CGC E Arg 595	Arg	GCC Ala	TTC Phe	ATC Ile	GCC Ala 600	GAG Glu	GAG Glu	GGG Gly	TGG Trp	CTA Leu 605	TTG Leu	GTG Val	GCC Ala	1944

	CTG Leu	GAC Asp 610	TAT Tyr	AGC Ser	CAG Gln	ATA Ile	GAG Glu 615	CTC Leu	AGG Arg	GTG Val	CTG Leu	GCC Ala 620	CAC His	CTC Leu	TCC Ser	GGC		1992
5	GAC Asp 625	GAG Glu	AAC Asn	CTG Leu	ATC Ile	CGG Arg 630	GTC Val	TTC Phe	CAG Gln	GAG Glu	GGG Gly 635	CGG Arg	GAC Asp	ATC Ile	CAC His	ACG Thr 640		2040
10	GAG Glu	ACC Thr	GCC Ala	AGC Ser	TGG Trp 645	ATG Met	TTC Phe	GGC Gly	GTC Val	CCC Pro 650	CGG Arg	GAG Glu	GCC Ala	GTG Val	GAC Asp 655	Pro		2088
15	CTG Leu	ATG Met	CGC Arg	CGG Arg 660	GCG Ala	GCC Ala	AAG Lys	ACC Thr	ATC Ile 665	AAC Asn	TTC Phe	GGG Gly	GTC Val	CTC Leu 670	Tyr	GGC Gly		2136
20	ATG Met	TCG Ser	GCC Ala 675	CAC His	CGC Arg	CTC Leu	TCC Ser	CAG Gln 680	GAG Glu	CTA Leu	GCC Ala	ATC Ile	CCT Pro 685	TAC Tyr	GAG Glu	GAG Glu		2184
20	GCC Ala	CAG Gln 690	GCC Ala	TTC Phe	ATT Ile	GAG Glu	CGC Arg 695	TAC Tyr	TTT Phe	CAG Gln	AGC Ser	TTC Phe 700	CCC Pro	AAG Lys	GTG Val	CGG Arg		2232
25	GCC Ala 705	TGG Trp	ATT Ile	GAG Glu	AAG Lys	ACC Thr 710	CTG Leu	GAG Glu	GAG Glu	GGC Gly	AGG Arg 715	AGG Arg	CGG Arg	GGG Gly	TAC Tyr	GTG Val 720		2280
30	GAG Glu	ACC Thr	CTC Leu	TTC Phe	GGC Gly 725	CGC Arg	CGC Arg	CGC Arg	TAC Tyr	GTG Val 730	CCA Pro	GAC Asp	CTA Leu	GAG Glu	GCC Ala 735	CGG Arg		2328
35	GTC Val	Lys	S AGO Ser	GTG Val 740	CGG Arg	GAG Glu	GCG Ala	GCC Ala	GAG Glu 745	CGC Arg	ATG Met	GCC	TTC Phe	AAC Asn 750	ATG Met	CCC Pro		2376
40	GTC Val	CAG Gln	GGC Gly 755	ACC Thr	GCC Ala	GCC Ala	GAC Asp	CTC Leu 760	ATG Met	AAG Lys	CTG Leu	GCT Ala	ATG Met 765	GTG Val	AAG Lys	CTC Leu		2424
40	TTC Phe	CCC Pro 770	AGG Arg	CTG Leu	GAG Glu	GAA Glu	ATG Met 775	GGG Gly	GCC Ala	AGG Arg	ATG Met	CTC Leu 780	CTT Leu	CAG Gln	GTC Val	CAC His	,	2472
45	GAC Asp 785	GAG Glu	CTG Leu	GTC Val	CTC Leu	GAG Glu 790	GCC Ala	CCA Pro	AAA Lys	GAG Glu	AGG Arg 795	GCG Ala	GAG Glu	GCC Ala	GTG Val	GCC Ala 800		2520
50	CGG Arg	CTG Leu	GCC Ala	AAG Lys	GAA Glu 805	GTC Val	ATG Met	GAG Glu	GGG Gly	GTG Val 810	TAT Tyr	CCC Pro	CTG Leu	GCC Ala	GTG Val 815	CCC Pro		2568
55												CTC Leu				GAG Glu		2616
	TGAT	racci	ACC															2626

60 (2) INFORMATION FOR SEQ ID NO::6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 amino acids

49

(B) TYPE: amino acid (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5		()	ci) S	EQUE	ENCE	DESC	CRIPT	CION	SEÇ	Q ID	NO:	:6:			•	
	Met 1	Arg	Gly	Met	Leu 5	Pro	Leu	Phe	Glu	Pro 10	Lys	Gly	Arg	Val	Leu 15	Leu
10	Val	Asp	Gly	His 20	His	Leu	Ala	Tyr	Cys 25	Thr	Phe	His	Ala	Leu 30	Lys	Gly
15	Leu	Thr	Thr 35	Ser	Arg	Gly	Glu	Pro 40	Val	Gln	Ala	Val	Tyr 45	Gly	Phe	Ala
	Lys	Ser 50	Leu	Leu	Lys	Ala	Leu 55	Lys	Glu	Asp	Gly	Asp 60	Ala	Val	Ile	Val
20	Val 65	Phe	Asp	Ala	Lys	Ala 70	Pro	Ser	Phe	Arg	His 75	Glu	Ala	Tyr	Gly	Gly 80
25	Tyr	Lys	Ala	Gly	Arg 85	Ala	Pro	Thr	Pro	Glu 90	Asp	Phe	Pro	Arg	Gln 95	Leu
23	Ala	Leu	Île	Lys 100	Gļu	Leu	Val	Asp	Leu 105	Leu	Gly	Leu	Ala	Arg 110	Leu	Glu
30	Val	Pro	Gly 115	Tyr	Glu	Ala	Asp	Asp 120	Val	Leu	Ala	Ser	Leu 125	Ala	Lys	Lys
	Ala	Glu 130	Lys	Glu	Gly	Tyr	Glu 135	Val	Arg	Ile	Leu	Thr 140	Ala	Asp	Lys	Asp
35	Leu 145	Tyr	Gln	Leu	Leu	Ser 150	Asp	Arg	Ile	His	Val 155	Leu	His	Pro	Glu	Gly 160
40	Tyr	Leu	Ile	Thr	Pro 165	Ala	Trp	Leu	Trp	Glu 170	Lys	Tyr	Gly	Leu	Arg 175	Pro
••	yab	Gln	Trp	Ala 180	Asp	Tyr	Arg	Ala	Leu 185	Thr	Gly	Asp	Glu	Ser 190	Asp	Asn
45	Leu	Pro	Gly 195	Val	Lys	Gly	Ile	Gly 200	Glu	Lys	Thr	Ala	Arg 205	Lys	Leu	Leu
	Glu	Glu 210	Trp	Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	Asn 220	Leu	Asp	Arg	Leu
50	Lys 225	Pro	Ala	Ile	Arg	Glu 230	Lys	Ile	Leu	Ala	His 235	Met	Asp	Asp	Leu	Lys 240
55	Leu	Ser	Trp	Asp	Leu 245	Ala	Lys	Val	Arg	Thr 250	Asp	Leu	Pro	Leu	Glu 255	Val
,,,	Asp	Phe	Ala	Lys 260	Arg	Arg	Glu	Pro	Asp 265	Arg	Glu	Arg	Leu	Arg 270	Ala	Ile
50	Le	ı Glu	275	g Lev	u Glu	ı Phe	e Gly	7 Ser 280	Pro	Let	Hi:	s Glu	285	e Gly	, Le	. Lev
	Glu	Ser 290	Pro	Lys	Ala	Leu	Glu 295	Glu	Ala	Pro	Trp	Pro 300	Pro	Pro	Glu	Gly

										-						
	Ala 305	Phe	Val	Gly	Phe	Val 310	Leu	Ser	Arg	Lys	Glu 315	Pro	Met	Trp	Ala	Asp 320
5	Leu	Leu	Ala	Leu	Ala 325	Ala	Ala	Arg	Gly	Gly 330	Arg	Val	His	Arg	Ala 335	Pro
	Glu	Pro	Tyr	Lys 340	Ala	Leu	Arg	Asp	Leu 345	Lys	Glu	Ala	Arg	Gly 350	Leu	Leu
10	Ala	Lys	Asp 355	Leu	Ser	Val	Leu	Ala 360	Leu	Arg	Glu	Gly	Leu 365	Gly	Leu	Pro
	Pro	Gly 370	Yab	Asp	Pro	Met	Leu 375	Leu	Ala	Tyr	Leu	Leu 380	Asp	Pro	Ser	Asn
15	Thr 385	Thr	Pro	Glu	Gly	Val 390	Ala	Arg	Arg	Tyr	Gly 395	Gly	Glu	Trp	Thr	Glu 400
20	Glu	Ala	Gly	Glu	Arg 405	Ala	Ala	Leu	Ser	Glu 410	Arg	Leu	Phe	Ala	Asn 415	Leu
	Trp	Gly	Arg	Leu 420	Glu	Gly	Glu	Glu	Arg 425	Leu	Leu	Trp	Leu	Tyr 430	Arg	Glu
25	Val	Glu	Arg 435	Pro	Leu	Ser	Ala	Val 440	Leu	Ala	His	Met	Glu 445	Ala	Thr	Gly
	Val	Arg 450	Leu	Asp	Val	Ala	Tyr 455	Leu	Arg	Ala	Leu	ser 460	Leu	Glu	Val	Ala
30	Glu 465	Glu	Ile	Ala	Arg	Leu 470	Glu	Ala	Glu	Val	Phe 475	Arg	Leu	Ala	Gly	His 480
35	Pro	Phe	Asn	Leu	Asn 485	Ser	Arg	Asp	Gln	Leu 490	Glu	Arg	Val	Leu	Phe 495	Asp
	Glu	Leu	Gly	Leu 500	Pro	Ala	Ile	Gly	Lys 505	Thr	Glų	Lys	Thr	Gly 510	Lys	Arg
40	Ser	Thr	Ser 515	Ala	Ala	Val	Leu	Glu 520	Ala	Leu	Arg	Glu	Ala 525	His	Pro	lle
	Val	Glu	Lys	Ile	Leu	Gln		Arg	Glu	Leu	Thr	Lys	Leu	Lys	Ser	Thr
45	Tyr 54 5	530 Ile	Asp	Pro	Leu	Pro 550	Asp	Leu	Ile	His	Pro 555	540 Arg	Thr	Gly	Arg	Leu 560
	His	Thr	Arg	Phe	Asn 56		Thr	Ala	Thr	Ala 570	Thr O	Ġly	Arg	Leu	Ser 579	Ser
50	Ser	Asp	Pro	Asn 580	Leu	Gln	Asn	Ile	Pro 585	Val	Arg	Thr	Pro	Leu 590	Gly	Gln
55	Arg	Ile	Arg 595	Arg	Ala	Phe	Ile	Ala 600	Glu	Glu	Gly	Trp	Leu 605	Leu	Val	Ala
	Leu	Asp 610	Tyr	Ser	Gln	Ile	Glu 615	Leu	Arg	Val	Leu	Ala 620	His	Leu	Ser	Gly
60	Asp 625		Asn	Leu		Arg 630	Val	Phe	Gln	Glu	Gly 635	Arg	Asp	Ile	His	Thr 640

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	Glu	Thr	Ala	Ser	Trp 645	Met	Phe	Gly	Val	Pro 650	Arg	Glu	Ala	Val	Asp 655	Pro		
5	Leu	Met	Arg	Arg 660	Ala	Ala	Lys	Thr	11e 665	Asn	Phe	Gly	Val	Leu 670		Gly	,	
	Met	Ser	Ala 675	His	Arg	Leu	Ser	Gln 680	Glu	Leu	Ala	Ile	Pro 685	Tyr	Glu	Glu		
10	Ala	Gln 690	Ala	Phe	Ile	Glu	Arg 695	Tyr	Phe	Gln	Ser	Phe 700	Pro	Lys	Val	Arg		
15	Ala 705	Trp	Ile	Glu	Lys	Thr 710	Leu	Glu	Glu	Gly	Arg 715	Arg	Arg	Gly	Tyr	Val 720		
13	Glu	Thr	Leu	Phe	Gly 725	Arg	Arg	Arg	Tyr	Val 730	Pro	Asp	Leu	Glu	Ala 735	Arg		
20	Val	Lys	Ser	Val 740	Arg	Glu	Ala	Ala	Glu 745	Arg	Met	Ala	Phe	Asn 750	Met	Pro		
	Val	Gln	Gly 755	Thr	Ala	Ala	Asp	Leu 760	Met	Lys	Leu	Ala	Met 765	Val	Lys	Leu		
25	Phe	Pro 770	Arg	Leu	Glu	Glu	Met 775	Gly	Ala	Arg	Met	Leu 780	Leu	Gln	Val	His		
30	785	Glu				790					795					800		
	_	Leu		_	805					810					815			
35	Leu	Glu	Val	Glu 820	Val	Gly	Ile	Gly	Glu 825	Asp	Trp	Leu	Ser	Ala 830	Lys	Glu		
	(2)	INFO	ORMA	NOI	FOR	SEQ	ID 1	10::3	7:									
40		(i)	(2 (1 (0	A) LI B) T) C) S?	engti (Pe : [rani	i: 26 nucl	TERI 526 h Leic ESS: line	ase acio sino	pair 1	:s								
45		(ii)	MOI	LECUI	LE TY	PE:	DNA	(ger	nomic	=)								
	1	(iii)	HYI	POTHI	ETIC	AL: 1	10											
50		(iv)	'MA	rı-sı	ENSE:	NO.								-				
		(vi)	OR:	IGINZ A) OI	AL SO RGANI	OURCE SM:	E: The:	cmus	aqua	iticu	15							
55		(ix)	(1	B) LO	AME/I	ON:	muta repl	lace	(89,	"g")) >= *	Thic	mıı	+ > + i i	.	results	in	a
	nuc	leot:		<i>)</i> (_
60					pol		case									DNA		
		(ix) FE	ATURI	Ξ:													

5	 (A) NAME/KEY: mutation (B) LOCATION: replace(934, "a") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 934 of the native T DNA polymerase nucleotide sequence of T.to A. This results in an amino acid change of Phe to Ile." 	'aq
10	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(962, "c") (D) OTHER INFORMATION: /note= "This mutation results in a</pre>	ʻaq
20	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(2535, "a") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 2535 of the native Town polymerase nucleotide sequence of G to A. This</pre>	ſaq
Ź5	mutation is conservative." (ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(341, "a") (D) OTHER INFORMATION: /note= "This mutation results in a	
30	nucleotide alteration at position 341 of the native T DNA polymerase nucleotide sequence of G to A. This mutation results in an amino acid change of Arg to His	
	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1212619	
35	<pre>(ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 1212616</pre>	
40	<pre>(ix) FEATURE: (A) NAME/KEY: - (B) LOCATION: 12619 (D) OTHER INFORMATION: /note= "pTarf5"</pre>	
45	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::7:	
	AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA	60
50	GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC	120
	ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15	168
55		216
60	CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala	264

									•							
	AAG AGG Lys Se:	Leu	CTC Leu	AAG Lys	GCC Ala	CTC Leu 55	AAG Lys	GAG Glu	GAC Asp	GGG Gly	GAC Asp 60	Ala	GTG Val	ATC Ile	GTG Val	312
5	GTC TT: Val Pho 65	GAC	GCC Ala	AAG Lys	GCC Ala 70	CCC Pro	TCC Ser	TTC Phe	CAC His	CAC His 75	GAG Glu	GCC Ala	TAC Tyr	GLY GLY	GGG Gly 80	360
10	TAC AAC Tyr Lys	G GCG B Ala	GGC Gly	CGG Arg 85	GCC Ala	CCC Pro	ACG Thr	CCG Pro	GAG Glu 90	Asp GAC	TTT Phe	CCC Pro	CGG Arg	CAA Gln 95	Leu	408
15	GCC CTC Ala Le	ATC 1 lle	AAG Lys 100	GAG Glu	CTG Leu	GTG Val	GAC Asp	CTC Leu 105	CTG Leu	GGG Gly	CTG Leu	GCG Ala	CGC Arg 110	Leu	GAG Glu	456
20	GTC CCC Val Pro	G GGC Gly 115	TAC Tyr	GAG Glu	GCG Ala	GAC Asp	GAC Asp 120	GTC Val	CTG Leu	GCC Ala	AGC Ser	CTG Leu 125	GCC Ala	AAG Lys	AAG Lys	504
20	GCG GAI Ala Gli 130	Lys	GAG Glu	GGC Gly	TAC Tyr	GAG Glu 135	GTC Val	CGC Arg	ATC Ile	CTC Leu	ACC Thr 140	GCC Ala	Aap Aap	AAA Lys	GAC Asp	552
25	CTT TAG Leu Ty: 145	C CAG	CTC Leu	CTT	TCC Ser 150	GAC Asp	CGC Arg	ATC Ile	CAC His	GTC Val 155	CTC Leu	CAC His	CCC Pro	GAG Glu	GGG Gly 160	600
30	TAC CTO	ATC La Ile	ACC Thr	CCG Pro 165	GCC Ala	TGG Trp	CTT Leu	TGG Trp	GAA Glu 170	AAG Lys	TAC Tyr	GGC Gly	CTG Leu	AGG Arg 175	Pro	648
35	GAC CAC	TGG Trp	GCC Ala 180	GAC Asp	TAC Tyr	CGG Arg	GCC Ala	CTG Leu 185	ACC Thr	GGG Gly	GAC Asp	GAG Glu	TCC Ser 190	Asp	AAC Asn	696
40	CTT CC	GGG Gly 195	GTC Val	AAG Lys	GGC Gly	ATC Ile	GGG Gly 200	GAG Glu	AAG Lys	ACG Thr	GCG Ala	AGG Arg 205	AAG Lys	CTT Leu	CTG Leu	744
40	GAG GAG Glu Gl 21	u Trp	GGG Gly	AGC Ser	CTG Leu	GAA Glu 215	GCC Ala	CTC Leu	CTC Leu	AAG Lys	AAC Asn 220	CTG Leu	GAC Asp	CGG Arg	CTG Leu	792
45	AAG CCC Lys Pro 225	C GCC	ATC Ile	Arg	GAG Glu 230	Lys	Ile	Leu	Ala	His	Met	Asp	Asp	Leu	Lys	840
50	CTC TC Leu Se	C TGG r Trp	GAC Asp	CTG Leu 245	GCC Ala	AAG Lys	GTG Val	CGC Arg	ACC Thr 250	GAC Asp	CTG Leu	CCC Pro	CTG Leu	GAG Glu 255	Val	888
55	GAC TT Asp Ph	C GCC e Ala	AAA Lys 260	Arg	CGG Arg	GAG Glu	CCC Pro	GAC Asp 265	CGG Arg	GAG Glu	AGG Arg	CTT Leu	AGG Arg 270	Ala	ATT Ile	936
	CTG GA Leu Gl	G AGG u Arg 275	Leu	GAG Glu	TTT Phe	GGC Gly	AGC Ser 280	CCC Pro	CTC Leu	CAC His	GAG Glu	TTC Phe 285	GGC Gly	CTT Leu	CTG Leu	984
60	GAA AG Glu Se 29	r Pro	AAG Lys	GCC Ala	CTG Leu	GAG Glu 295	GAG Glu	GCC Ala	CCC Pro	TGG Trp	CCC Pro 300	CCG Pro	CCG Pro	GAA Glu	GGG Gly	1032

	GCC Ala 305	TTC Phe	GTG Val	GGC Gly	TTT Phe	GTG Val 310	CTT Leu	TCC Ser	CGC Arg	AAG Lys	GAG Glu 315	CCC Pro	ATG Met	TGG Trp	GCC Ala	GAT Asp 320	1080
5	CTT Leu	CTG Leu	GCC Ala	CTG Leu	GCC Ala 325	GCC Ala	GCC Ala	AGG Arg	GGG Gly	GGC Gly 330	Arg	GTC Val	CAC His	CGG Arg	GCC Ala 335	CCC Pro	1128
10	GAG Glu	CCT Pro	TAT Tyr	AAA Lys 340	GCC Ala	CTC Leu	AGG Arg	GAC Asp	CTG Leu 345	AAG Lys	GAG Glu	GCG Ala	CGG Arg	GGG Gly 350	Leu	CTC Leu	1176
15	GCC Ala	AAA Lys	GAC Asp 355	CTG Leu	AGC Ser	GTT Val	CTG Leu	GCC Ala 360	CTG Leu	AGG Arg	GAA Glu	GGC Gly	CTT Leu 365	GGC Gly	CTC Leu	CCG Pro	1224
20	CCC Pro	GGC Gly 370	GAC Asp	GAC Asp	CCC Pro	ATG Met	CTC Leu 375	CTC Leu	GCC Ala	TAC Tyr	CTC Leu	CTG Leu 380	GAC Asp	CCT Pro	TCC Ser	AAC Asn	1272
20	ACC Thr 385	ACC Thr	CCC Pro	GAG Glu	GGG Gly	GTG Val 390	GCC Ala	CGG Arg	CGC Arg	TAC Tyr	GGC Gly 395	GGG Gly	GAG Glu	TGG Trp	ACG Thr	GAG Glu 400	1320
25	GAG Glu	GCG Ala	GGG	GAG Glu	CGG Arg 405	GCC Ala	GCC Ala	CTT Leu	TCC Ser	GAG Glu 410	AGG Arg	CTC Leu	TTC Phe	GCC Ala	AAC Asn 415	CTG Leu	1368
30	TGG Trp	GGG Gly	AGG Arg	CTT Leu 420	Glu	GGG Gly	GAG Glu	GAG Glu	AGG Arg 42	Leu	CTT Leu	TGG Trp	CTT Leu	TAC Tyr 43	Arg	GAG Glu	1416
35	GTG Val	GAG Glu	AGG Arg 435	CCC Pro	CTT Leu	TCC Ser	GCT Ala	GTC Val 440	CTG Leu	GCC Ala	CAC His	ATG Met	GAG Glu 445	GCC Ala	ACG Thr	GGG Gly	1464
40	GTG Val	CGC Arg 450	CTG Leu	GAC Asp	GTG Val	GCC Ala	TAT Tyr 455	CTC Leu	AGG Arg	GCC Al.a	TTG Leu	TCC Ser 460	CTG Leu	GAG Glu	GTG Val	GCC Ala	1512
40	GAG Glu 465	GAG Glu	ATC Ile	GCC Ala	CGC Arg	CTC Leu 470	GAG Glu	GCC Ala	GAG Glu	GTC Val	TTC Phe 475	CGC Arg	CTG Leu	GCC Ala	GGC Gly	CAC His 480	1560
45	CCC Pro	TTC Phe	AAC Asn	CTC Leu	AAC Asn 485	TCC Ser	CGG Arg	GAC Asp	CAG Gln	CTG Leu 490	GAA Glu	AGG Arg	GTC Val	CTC Leu	TTT Phe 495	GAC Asp	1608
50	GAG Glu	CTA Leu	GGG Gly	CTT Leu 500	CCC Pro	GCC Ala	ATC Ile	GGC Gly	AAG Lys 505	ACG Thr	GAG Glu	AAG Lys	ACC Thr	GGC Gly 510	AAG Lys	C GC A rg	1656
55	TCC Ser	ACC Thr	AGC Ser 515	GCC Ala	GCC Ala	GTC Val	CTG Leu	GAG Glu 520	GCC Ala	CTC Leu	CGC Arg	GAG Glu	GCC Ala 525	CAC His	CCC Pro	ATC Ile	1704
60	GTG Val	GAG Glu 530	AAG Lys	ATC Ile	CTG Leu	CAG Gln	TAC Tyr 535	CGG Arg	GAG Glu	CTC Leu	ACC Thr	AAG Lys 540	CTG Leu	AAG Lys	AGC Ser	ACC Thr	1752
00	TAC Tyr 54	Ile	GAC Asp	CCC Pro	TTG Leu	CCG Pro 550	Asp	CTC Leu	ATC Ile	CAC His	CCC Pro 55!	Arg	ACG Thr	GGC Gly	CGC Arg	CTC Leu 560	1800

	CAC ACC	CGC TTC Arg Phe	AAC CAG Asn Gln 565	ACG GCC Thr Ala	ACG GCC . Thr Ala 570	ACG GGC A Thr Gly 1	GG CTA AGT Arg Leu Ser 575	AGC 1848 Ser
5	TCC GAT Ser Asp	CCC AAC Pro Asn 580	Leu Gln	AAC ATC Asn Ile	CCC GTC Pro Val 585	CGC ACC C Arg Thr 1	CG CTT GGG Pro Leu Gly 590	CAG 1896 Gln
10	AGG ATC Arg Ile	CGC CGG Arg Arg 595	GCC TTC Ala Phe	ATC GCC Ile Ala 600	GAG GAG Glu Glu	Gly Trp I	TA TTG GTG Leu Leu Val 505	GCC 1944 Ala
15	CTG GAC Leu Asp 610	Tyr Ser	CAG ATA Gln Ile	GAG CTC Glu Leu 615	AGG GTG Arg Val	CTG GCC C Leu Ala F 620	AC CTC TCC His Leu Ser	GGC 1992 Gly
20	GAC GAG Asp Glu 625	AAC CTG Asn Leu	ATC CGG Ile Arg 630	GTC TTC Val Phe	Gln Glu	GGG CGG G Gly Arg # 635	AC ATC CAC Asp Ile His	ACG 2040 Thr 640
	GAG ACC Glu Thr	GCC AGC Ala Ser	TGG ATG Trp Met 645	TTC GGC Phe Gly	GTC CCC Val Pro 650	CGG GAG G Arg Glu <i>F</i>	CC GTG GAC Ala Val Asp 655	CCC 2088 Pro
25	CTG ATG Leu Met	CGC CGG Arg Arg 660	Ala Ala	AAG ACC Lys Thr	ATC AAC 1 1le Asn 665	TTC GGG G Phe Gly V	TC CTC TAC Val Leu Tyr 670	GGC 2136 Gly
30	ATG TCG Met Ser	GCC CAC Ala His 675	CGC CTC Arg Leu	TCC CAG Ser Gln 680	Glu Leu	GCC ATC C Ala Ile F	CT TAC GAG Pro Tyr Glu 685	GAG 2184 Glu
35	GCC CAG Ala Gln 690	Ala Phe	ATT GAG Ile Glu	CGC TAC Arg Tyr 695	TTT CAG A	AGC TTC C Ser Phe F 700	CC AAG GTG Pro Lys Val	CGG 2232 Arg
40	GCC TGG Ala Trp 705	ATT GAG Ile Glu	AAG ACC Lys Thr 710	CTG GAG Leu Glu	Glu Gly	AGG AGG C Arg Arg A 715	GG GGG TAC	GTG 2280 Val 720
70	GAG ACC Glu Thr	CTC TTC Leu Phe	GGC CGC Gly Arg 725	CGC CGC Arg Arg	TAC GTG (Tyr Val 730	CCA GAC C Pro Asp I	TA GAG GCC Leu Glu Ala 735	CGG 2328 Arg .
45	GTG AAG Val Lys	Ser Val	Arg Glu	Ala Ala	Glu Arg	ATG GCC T Met Ala F	TC AAC ATG Phe Asn Met 750	CCC 2376 Pro
50	GTC CAG Val Gln	GGC ACC Gly Thr 755	GCC GCC Ala Ala	GAC CTC Asp Leu 760	ATG AAG (Met Lys	Leu Ala M	TG GTG AAG Met Val Lys 165 -	CTC 2424 Leu
55	TTC CCC Phe Pro 770	Arg Leu	GAG GAA Glu Glu	ATG GGG Met Gly 775	GCC AGG A Ala Arg	ATG CTC C Met Leu I 780	TT CAG GTC Leu Gln Val	CAC 2472 His
60	GAC GAG Asp Glu 785	CTG GTC Leu Val	CTC GAG Leu Glu 790	GCC CCA Ala Pro	Lys Glu	AGG GCG G Arg Ala G 795	AG GCC GTG Glu Ala Val	GCC 2520 Ala 800
00						Tyr Pro I	TG GCC GTG Leu Ala Val 819	Pro

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										5 6							
	CTG Leu	GAG Glu	GTG Val	GAG Glu 820	GTG Val	GGG Gly	ATA Ile	GGG Gly	GAG Glu 825	Asp	TGG Trp	CTC Leu	TCC Ser	GCC Ala 830	Lys	GAG Glu	2616
5	TGA'	TACC	ACC												•		2626
	(2)	INF	ORMA	TION	FOR	SEQ	ID 1	NO::	8:								
10			(i)	(A (B	ENCE) LEI) TYI) TOI	NGTH PE: 6	: 83: amino	2 am.	ino id		8						
15		(:	ii) 1	MOLE	CULE	TYPI	e: p	rote	in								
		(:	xi) :	SEQUI	ENCE	DES	CRIP:	LION	: SE	Q ID	NO:	:8:					
20	Met 1	Arg	Gly	Met	Leu 5	Pro	Leu	Phe	Glu	Pro 10	Lys	Gly	Arg	Val	Leu 15	Leu	
	Val	Asp	Gly	His 20	His	Leu	Ala	Tyr	Arg 25	Thr	Phe	His	Ala	Leu 30	Lys	Gly	
25	Leu	Thr	Thr 35	Ser	Arg	Gly	Glu	Pro 40	Val	Gln	Ala	Val	Tyr 45	Gly	Phe	Ala	
30	Lys	Ser 50	Leu	Leu	Lys	Ala	Leu 55	Lys	Glu	Asp	Gly	Aap	Ala	Val	Ile	Val	
30	Val 65	Phe	Asp	Ala	Lys	Ala 70	Pro	Ser	Phe	His	His 75	Glu	Ala	Tyr	Gly	Gly 80	
35	Tyr	Lys	Ala	Gly	Arg 85	Ala	Pro	Thr	Pro	Glu 90	Asp	Phe	Pro	Arg	Gln 95	Leu	
	Ala	Leu	Ile	Lys 100	Glu	Leu	Val	Asp	Leu 105	Leu	Gly	Leu	Ala	Arg 110	Leu	Glu	
40	Val	Pro	Gly 115	Tyr	Glu	Äla	Asp	Asp 120	Val	Leu	Ala	Ser	Leu 125	Ala	Lys	Lys	
45	Ala	Glu 130	Lys	Glu	Gly	Tyr	Glu 135	Val	Arg	Ile	Leu	Thr 140	Ala	Asp	Lys	Asp	
13	Leu 145	Tyr	Gln	Leu	Leu	Ser 150	Asp	Arg	Ile	His	Val 155	Leu	His	Pro	Glu	Gly 160	
50	Tyr	Leu	Ile	Thr	Pro 165	Ala	Trp	Leu	Trp	Glu 170	Lys	Tyr	Gly	Leu	Arg 175	Pro	
	Asp	Gln	Trp	Ala 180	Asp	Tyr	Arg	Ala	Leu 185	Thr	Gly	Asp	Glu	Ser 190	Asp	Asn	
55	Leu	Pro	Gly 195	Val	Lys	Gly	Ile	Gly 200	Glu	Lys	Thr	Ala	Arg 205	Lys	Leu	Leu	
50	Glu	Glu 210	Trp	Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	Asn 220	Leu	Asp	Arg	Leu	
,,,	Lys 225	Pro	Ala	Ile	Arg	Glu 230	Lys	Ile	Leu	Ala	His 235	Met	Asp	Asp	Leu	Lys 240	

	Leu	Ser	Trp	qaA	Leu 245	Ala	Lys	Val	Arg	Thr 250	Asp	Leu	Pro	Leu	Glu 255	Val
5	Asp	Phe	Ala	Lys 260	Arg	Arg	Glu	Pro	Asp 265	Arg	Glu	Arg	Leu	Arg 270		Ile
	Leu	Glu	Arg 275	Leu	Glu	Phe	Gly	Ser 280	Pro	Leu	His	Glu	Phe 285	Gly	Leu	Leu
10	Glu	Ser 290	Pro	Lys	Ala	Leu	Glu 295	Glu	Ala	Pro	Trp	Pro 300	Pro	Pro	Glu	Gly
15	Ala 305	Phe	Val	Gly	Phe	Val 310	Leu	Ser	Arg	Lys	Glu 315	Pro	Met	Trp	Ala	Asp 320
15	Leu	Leu	Ala	Leu	Ala 325	Ala	Ala	Arg	Gly	Gly 330	Arg	Val	His	Arg	Ala 335	Pro
20	Glu	Pro	Tyr	Lys 340	Ala	Leu	Arg	Asp	Leu 345	Lys	Glu	Ala	Arg	Gly 350	Leu	Leu
	Ala	Lys	Asp 355	Leu	Ser	Val	Leu	Ala 360	Leu	Arg	Glu	Gly	Leu 365	Gly	Leu	Pro
25	Pro	Gly 370	Asp	Asp	Pro	Met	Leu 375	Leu	Ala	Tyr	Leu	Leu 380	Asp	Pro	Ser	Asn
30	Thr 385	Thr	Pro	Glu	Gly	Val 390	Ala	Arg	Arg	Tyr	Gly 395	Gly	Glu	Trp	Thr	Glu 400
30	Glu	Ala	Gly	Glu	Arg 405	Ala	Ala	Leu	Ser	Glu 410	Arg	Leu	Phe	Ala	Asn 415	Leu
35	Trp	Gly	Arg	Leu 420	Glu	Gly	Glu	Glu	Arg 425	Leu	Leu	Trp	Leu	Tyr 430	Arg	Glu
			435					440					445	Ala		
40	Val	Arg 450	Leu	Asp	Val	Ala	Tyr 455	Leu	Arg	Ala	Leu	Ser 460	Leu	Glu	Val	Ala
45	Glu 465	Glu	Ile	Ala	Arg	Leu 470	Glu	Ala	Glu	Val	Phe 475	Arg	Leu	Ala	Gly	His 480
	Pro	Phe	Asn	Leu	Asn 485	Ser	Arg	Asp	Gln	Leu 490	Glu	Arg	Val	Leu	Phe 495	Asp
50	Glu	Leu	Gly	Leu 500	Pro	Ala	Ile	Gly	Lys 505	Thr	Glu	Lys	Thr	Gly 510	Lys	Arg
	Ser	Thr	Ser 515	Ala	Ala	Val	Leu	Glu 520	Ala	Leu	Arg	Glu	Ala 525	His	Pro	Ile
55	Val	Glu 530	Lys	Ile	Leu	Gln	Tyr 535	Arg	Glu	Leu	Thr	Lys 540	Leu	Lys	Ser	Thr
60	545		-			550					555			Gly		560
	His	Thr	Arg	Phe	Asn 565	Gln	Thr	Ala	Thr	Ala 570	Thr	Gly	Arg	Leu	Ser 575	Ser

58

	Ser	Asp	Pro	Asn 580	Leu	Gln	Asn	Ile	Pro 585	Val	Arg	Thr	Pro	Leu 590	Gly	Gln
5	Arg	Ile	Arg 595	Arg	Ala	Phe	Ile	Ala 600	Glu	Glu	Gly	Trp	Leu 605	Leu	Val	Ala
	Leu	Asp 610	Tyr	Ser	Gln	lle	Glu 615	Leu	Arg	Val	Leu	Ala 620	His	Leu	Ser	Gly
10	Asp 625	Glu	Asn	Leu	Ile	Arg 630	Val	Phe	Gln	Glu	Gly 635	Arg	ysb	Ile	His	Thr 640
	Glu	Thr	Ala	Ser	Trp 645	Met	Phe	Gly	Val	Pro 650	Arg	Glu	Ala	Val	Asp 655	Pro
15	Leu	Met	Arg	Arg 660	Ala	Ala	Lys	Thr	Ile 665	Asn	Phe	Gly	Val	Leu 670	Tyr	Gly
20	Met	Ser	Ala 675	His	Arg	Leu	Ser	Gln 680	Glu	Leu	Ala	Ile	Pro 685	Tyr	Glu	Glu
	Ala	Gln 690	Ala	Phe	Ile	Glu	Arg 695	Tyr	Phe	Gln	Ser	Phe 700	Pro	Lys	Val	Arg
25	Ala 705	Trp	Ile	Glu	Lys	Thr 710	Leu	Glu	Glu	Gly	Arg 715	Arg	Arg	Gly	Tyr	Val 720
30	Glu	Thr	Leu	Phe	Gly 725	Arg	Arg	Arg	Tyr	Val 730	Pro	Asp	Leu	Glu	Ala 735	Arg
50	V a	l Ly:	s Se	740	l Ar	g Glu	Ala	a Ala	745	ı Arç	y Met	. Ala	a Phe	750	n Met	: Pro
35	Val	Gln	Gly 755	Thr	Ala	Ala	Asp	Leu 760	Met	Lys	Leu	Ala	Met 765	Val	Lys	Leu
	Phe	Pro 770	Arg	Leu	Glu	Glu	Met 775	Gly	Ala	Arg	Met	Leu 780	Leu	Gln	Val	His
40	Asp 785	Glu	Leu	Val	Leu	Glu 790	Ala	Pro	Lys	Glu	Arg 795	Ala	Glu	Ala	Val	Ala 800
4 E	Arg	Leu	Ala	Lys	Glu 805	Val	Met	Glu	Gly	Val 810	Tyr	Pro	Leu	Ala	Val 815	Pro
45	Leu	Glu	Val	Glu 820	Val	Gly	Ile	Gly	Glu 825	Asp	Trp	Leu	Ser	Ala 830	Lys	Glu
50	(2)	INF	ORMA	TION	FOR	SEQ	ID I	NO::	9:							
		(i	(A) L B) T	CE C ENGT: YPE:	H: 2	626 leic	base aci	pai: d	rs			-			
55			(C) S D) T	TRAN OPOL	OGY:	lin	ear	gre							
		(ii) MO	LECU	LE T	YPE:	DNA	(ge	nomi	c)						
60		(iii) HY	ротн	ETIC	AL:	МО									

(ix) FEATURE:
(A) NAME/KEY: mutation

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	(B) LOCATION: replace(89, "g") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at postion 89 of the native Taq DNA polymerase nucleotide sequence of C to G."
5	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(934, "a") (D) OTHER INFORMATION: /note= "This mutation results in a</pre>
10	nucleotide alteration at position 934 of the native Tag DNA polymerase nucleotide sequence of T to A. This results in an amino acid change of Phe to Ile."
15 .	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(962, "c") (D) OTHER INFORMATION: /note= "This mutation results in a</pre>
20	results in an amino acid change of Leu to Pro."
25	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(2535, "a") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 2535 of the native Tag</pre>
	DNA polymerase nucleotide sequence of G to A. This mutation is conservative."
30	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(337, "a") (D) OTHER INFORMATION: /note= "This mutation results in a</pre>
35	nucleotide alteration at position 337 of the native Taq DNA polymerase nucleotide sequence of T to C. This change results in an amino acid change of Phe to Leu."
40	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1212619
45	<pre>(ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 1212616</pre>
43	(ix) FEATURE: (A) NAME/KEY: - (B) LOCATION: 12619
50	(D) OTHER INFORMATION: /note= "pTarf2"
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::9:
55	AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA 60
	GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC 120
60	ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15
	GTG GAC GGC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC 216

	Val	Asp	Gly	His 20	His	Leu	Ala	Tyr	Arg 25	Thr	Phe	His	Ala	Leu 30	Lys	Gly		
5	CTC Leu	ACC Thr	ACC Thr 35	AGC Ser	CGG Arg	GGG Gly	GAG Glu	CCG Pro 40	GTG Val	CAG Gln	GCG Ala	GTC Val	TAC Tyr 45	GGC Gly	TTC Phe	GCC Ala	:	264
10	Lys	Ser 50	Leu	Leu	Lys	Ala	Leu 55	Lys	GAG Glu	Asp	GIY	60	Ala	Agi	116	vai		312
	GTC Val 65	TTT Phe	GAC Asp	GCC Ala	AAG Lys	GCC Ala 70	CCC Pro	TCC Ser	CTC Leu	CGC Arg	CAC His 75	GAG Glu	GCC Ala	TAC Tyr	GGG Gly	GGG 80	:	360 _.
15	TAC Tyr	AAG Lys	GCG Ala	GGC Gly	CGG Arg 85	GCC Ala	CCC Pro	ACG Thr	CCG Pro	GAG Glu 90	Asp Asp	TTT Phe	CCC Pro	CGG Arg	CAA Gln 95	CTC Leu		408
20	GCC Ala	CTC Leu	ATC Ile	AAG Lys 100	GAG Glu	CTG Leu	GTG Val	GAC Asp	CTC Leu 105	CTG Leu	GGG Gly	CTG Leu	GCG Ala	CGC Arg 110	CTC Leu	GAG Glu		456
25	GTC Val	CCG Pro	GGC Gly 115	TAC Tyr	GAG Glu	GCG Ala	GAC Asp	GAC Asp 120	GTC Val	CTG Leu	GCC Ala	AGC Ser	CTG Leu 125	GCC Ala	AAG Lys	AAG Lys		504
30	GCG Al	GAA a Gl	u Ly	GAG s Gl	GGC u Gl	TAC y Ty	GAG r Gl 135	GTC u Va	CGC 1 Ar	ATC g Il	CTC e Le	ACC u Th: 140	GCC r Al	GAC a As	AAA p Ly	a Asp		552
	CTT Leu 145	Tyr	CAG Gln	CTC Leu	CTT Leu	TCC Ser 150	GAC Asp	CGC Arg	ATC Ile	CAC His	GTC Val 155	Leu	CAC His	CCC Pro	GAG Glu	GGG Gly 160		600
35	TAC Tyr	CTC Leu	ATC Ile	ACC	CCG Pro 165	Ala	TGG Trp	CTT Leu	TGG Trp	GAA Glu 170	rys	TAC Tyr	GGC Gly	CTG Leu	AGG Arg 175	PIU		648
40	GAC Asp	CAG Gln	TGG Trp	GCC Ala 180	Asp	TAC Tyr	CGG Arg	GCC Ala	CTG Leu 185	Thr	GGG Gly	GAC Asp	GAG Glu	TCC Ser 190	ASP	AAC Asn	•	696
45	CTT Leu	CCC Pro	GGG Gly 195	Val	AAG Lys	GGC Gly	ATC Ile	GGG Gly 200	GIU	AAG Lys	ACG Thr	GCG Ala	AGG Arg 205	гÃа	CTT Leu	CTG Leu		744
50	GAG Glu	Glu	Trp	GGG Gly	AGC Ser	CTG Leu	GAA Glu 215	Ala	CTC Leu	CTC Leu	AAG Lys	AAC Asn 220	Leu	GAC Asp	CGG Arç	CTG Leu		792
	AAG Lys 225	Pro	GCC Ala	ATC	CGG	GAG Glu 230	Lys	ATC Ile	CTG Leu	GCC Ala	CAC His 235	, wet	GAC Asp	GAT Asp	CTG Lev	AAG Lys 240		840
55	CTC	TCC Ser	TGG	GAC Asp	CTG Leu 245	Ala	AAG Lys	GTG Val	CGC Arg	ACC Thr 250	Asp	CTG Leu	CCC Pro	CTG Leu	GAG Glu 255	GTG Val		888
60	GAC Asp	TTC Phe	GCC Ala	AAA Lys 260	Arc	CGG Arg	GAG Glu	CCC Pro	GAC Asp 265	Arc	GAG Glu	AGG Arg	CTT Lev	AGG Arg 270	ATS	ATT a Ile		936

	CTG Leu	GAG Glu	AGG Arg 275	CTT Leu	GAG Glu	TTT Phe	GGC Gly	AGC Ser 280	Pro	CTC Leu	CAC His	GAG Glu	TTC Phe 285	Gly	CTT Lev	CTG Leu		984
5	GAA Glu	AGC Ser 290	CCC Pro	AAG Lys	GCC Ala	CTG Leu	GAG Glu 295	GAG Glu	GCC Ala	CCC Pro	TGG Trp	CCC Pro 300	Pro	CCG Pro	GAA Glu	GGG Gly		1032
10	GCC Ala 305	TTC Phe	GTG Val	GGC Gly	TTT Phe	GTG Val 310	CTT Leu	TCC Ser	CGC Arg	AAG Lys	GAG Glu 315		ATG Met	TGG Trp	GCC Ala	GAT Asp 320	•	1080
15	CTT Leu	CTG Leu	GCC Ala	CTG Leu	GCC Ala 325	GCC Ala	GCC Ala	AGG Arg	GGG Gly	GGC Gly 330	Arg	GTC Val	CAC His	CGG Arg	GCC Ala 335	Pro	•	1128
20	GAG Glu	CCT Pro	TAT Tyr	AAA Lys 340	GCC Ala	CTC Leu	AGG Arg	GAC Asp	CTG Leu 345	AAG Lys	GAG Glu	GCG Ala	CGG Arg	GGG Gly 350	Leu	CTC Leu		1176
20	GCC Ala	AAA Lys	GAC Asp 355	CTG Leu	AGC Ser	GTT Val	CTG Leu	GCC Ala 360	CTG Leu	AGG Arg	GAA Glu	GGC Gly	CTT Leu 365	GGC Gly	CTC Leu	CCG Pro		1224
25												CTG Leu 380						1272
30	ACC Thr 385	ACC Thr	CCC Pro	GAG Glu	GGG Gly	GTG Val 390	GCC Ala	CGG Arg	CGC Arg	TAC Tyr	GGC Gly 395	GGG Gly	GAG Glu	TGG Trp	ACG Thr	GAG Glu 400		1320
35	GAG Glu	GCG Ala	GGG Gly	GAG Glu	CGG Arg 405	GCC Ala	GCC Ala	CTT Leu	TCC Ser	GAG Glu 410	AGG Arg	CTC Leu	TTC Phe	GCC Ala	AAC Asn 415	CTG Leu		1368
40	TGG Trp	GGG Gly	AGG Arg	CTT Leu 420	GAG Glu	GGG Gly	GAG Glu	GAG Glu	AGG Arg 425	CTC Leu	CTT Leu	TGG Trp	CTT Leu	TAC Tyr 430	CGG Arg	GAG Glu		1416
	GTG Val	GAG Glu	AGG Arg 435	CCC Pro	CTT Leu	TCC Ser	GCT Ala	GTC Val 440	CTG Leu	GCC Ala	CAC His	ATG Met	GAG Glu 445	GCC Ala	ACG Thr	GGG Gly		1464
45	Val	Arq	Leu	Asp	Val	Ala	Tyr	Leu	Arg	Ala	Leu	TCC Ser 460	Leu					1512
50												CGC Arg						1560
55												AGG Arg						1608
	GAG Glu	CTA Leu	GGG Gly	CTT Leu 500	CCC Pro	GCC Ala	ATC Ile	GGC Gly	AAG Lys 505	ACG Thr	GAG Glu	AAG Lys	ACC Thr	GGC Gly 510	AAG Lys	CGC Arg		1656
60												GAG (Glu						1704

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	GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr 530 540	1752
5	TAC ATT GAC CCC TTG CCG GAC CTC ATC CAC CCC AGG ACG GGC CGC CTC Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu 545 550 555 560	1800
10	CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser 565 570 575	1848
15	TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln 580 585 590	1896
20	AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala 595 600 605	1944
20	CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly 610 615 620	1992
25	GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr 625 630 635 640	2040
30	GAG ACC GCC AGC TGG ATG TTC GGC GTC CCC CGG GAG GCC GTG GAC CCC Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro 645 650 655	2088
35	CTG ATG CGC CGG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly 660 665 670	2136
40	ATG TCG GCC CAC CGC CTC TCC CAG GAG CTA GCC ATC CCT TAC GAG GAG Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu 675 680 685	2184
40	GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC TTC CCC AAG GTG CGG Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg 690 695 700	2232
45	GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val 705 710 715 720	2280
50	GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG Glu Thr Leu Phe Gly Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg 725 730 735	2328
55	GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro 740 745 750	2376
	GTC CAG GGC ACC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu 755 760 765	2424
60	TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His 770 780	2472

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	GAC Asp 785	Glu	G CTO	G GTC	CTC Leu	GAG Glu 790	Ala	CCA Pro	AAA D Lys	GAG Glu	AGG Arg 795	g Ala	GAG Glu	GCC Ala	GTG a Va	GCC 1 Ala 800	2520
5	CGG Arg	Leu	G GCC	AAG Lys	GAA Glu 805	Val	ATG Met	GAG Glu	GGG Gly	GTG Val 810	Tyz	CCC Pro	CTG Lev	GCC Ala	GTG Val 815	l Pro	2568
10	CTG Leu	GAG Glu	GTG Val	GAG Glu 820	val	GGG Gly	ATA Ile	GGG Gly	GAG Glu 825	Asp	TGG Trp	CTC Lev	TCC Ser	GCC Ala 830	Lys	GAG Glu	2616
	TGA	TACC	CACC														2626
15																	
	(2)	INF	ORMA	TION	FOR	SEQ	ID	No::	10:								
20				(A (B (D	ENCE) LE) TY	NGTH PE: POLO	: 83 amin GY:	2 am o ac line	ino id ar		s						
		(77)	MOLE	CULE	TYP	e: p	rote	in								
25		(xi)	SEQU	ENCE	DES	CRIP	TION	: SE	Q ID	NO:	:10:					
	Met 1	Arg	Gly	Met	Leu 5	Pro	Leu	Phe	Glu	Pro 10	Lys	Gly	Arg	Val	Leu 15	Leu	
30	Val	Asp	Gly	His 20	His	Leu	Ala	Tyr	Arg 25	Thr	Phe	His	Ala	Leu 30	Lys	Gly	
35			35					40			٠		Tyr 45	_			
		50					55					60	Ala				
40	65					70					75		Ala			80	
4.5					85			• •		90			Pro	_	95		
45				100					105				Ala	110			
50			115					120					Leu 125			_	
		130					135					140	Ala	_		-	
55	145					150					155		His		•	160	
					165					170			Gly		175		
60	Asp	Gln	Trp	Ala 180	Asp	Tyr	Arg	Ala	Leu 185	Thr	Gly	Asp		Ser 190	Asp	Asn	

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	Leu	Pro	Gly 195		Lys	Gly	Ile	Gly 200		Lys	Thr	Ala	Arg 205		Leu	Le
, 5	Glu	Glu 210		Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	220		Asp	Arg	Let
	Lys 225	Pro	Ala	Ile	Arg	Glu 230		Ile	Leu	Ala	His 235		Asp	yat	Leu	Lys 240
10	Leu	Ser	Trp	Asp	Leu 245	Ala	Lys	Val	Arg	Thr 250		Leu	Pro	Leu	Glu 255	
15	Asp	Phe	Ala	Lys 260		Arg	Glu	Pro	Авр 265		Glu	Arg	Leu	Arg 270		Ile
	Leu	Glu	Arg 275	Leu	Glu	Phe	Gly	ser 280	Pro	Leu	His	Glu	Phe 285	Gly	Leu	Leu
20	Glu	Ser 290	Pro	Lys	Ala	Leu	Glu 295	Glu	Ala	Pro	Trp	Pro 300		Pro	Glu	Gly
	Ala 305	Phe	Val	Gly	Phe	Val 310	Leu	Ser	Arg	Lys	Glu 315	Pro	Met	Trp	Ala	Asp 320
25	Leu	Leu	Ala	Leu	Ala 325	Ala	Ala	Arg	Gly	Gly 330		Val	His	Arg	Ala 335	Pro
30	Glu	Pro	Tyr	Lys 340	Ala	Leu	Arg	Asp	Leu 345	Lys	Glu	Ala	Arg	Gly 350	Leu	Leu
	Ala	a Lys	355	p Let	ı Sei	· Val	l Lei	360	a Le	u Ar	g Glu	ı Gl	y Lei 365	u Gl	y Lei	ı Pr
35	Pro	Gly 370	Asp	Asp	Pro	Met	Leu 375	Leu	Ala	Tyr	Leu	Leu 380	Asp	Pro	Ser	Asn
	Thr 385	Thr	Pro	Glu	Gly	Val 390		Arg	Arg	Tyr	Gly 395	Gly	Glu	Trp	Thr	Glu 400
40	Glu	Ala	Gly	Glu	Arg 405	Ala	Ala	Leu	Ser	Glu 410	Arg	Leu	Phe	Ala	Asn 415	Leu
45	Trp	Gly	Arg	Leu 420	Glu	Gly	Glu	Glu	Arg 425	Leu	Leu	Trp	Leu	Tyr 430	Arg	Glu
	Val	Glu	Arg 435	Pro	Leu	Ser	Ala	Val 440	Leu	Ala	His	Met	Glu 445	Ala	Thr	Gly
50	Val	Arg 450	Leu	Asp	Val	Ala	Tyr 455	Leu	Arg	Ala	Leu	Ser 460	Leu	Glu	Val	Ala
	Glu 465	Glu	Ile	Ala	Arg	Leu 470	Glu	Ala	Glu	Val	Phe 475	Arg	Leu	Ala	Gly	His 480
55	Pro	Phe	Asn	Leu	Asn 485	Ser	Arg	Asp	Gln	Leu 490	Glu	Arg	Val	Leu	Phe 495	Asp
60				500				Gly	505					510	_	_
	Ser	Thr	Ser 515	Ala	Ala	Val	Leu	Glu 520	Ala	Leu	Arg	Glu	Ala 525	His	Pro	Ile

	Val	Glu 530	Lys	Ile	Leu	Gln	Tyr 535	Arg	Glu	Leu	Thr	Lys 540	Leu	Lys	Ser	Thr
5	Tyr 545	Ile	Asp	Pro	Leu	Pro 550	yab	Leu	Ile	His	Pro 555	Arg	Thr	Gly	Arg	Leu 560
	His	Thr	Arg	Phe	Asn 565	Gln	Thr	Ala	Thr	Ala 570	Thr	Gly	Arg	Leu	Ser 575	Ser
10	Ser	Asp	Pro	Asn 580	Leu	Gln	Asn	Ile	Pro 585	Val	Arg	Thr	Pro	Leu 590	Gly	Gln
15	Arg	Ile	Arg 595	Arg	Ala	Phe	Ile	Ala 600	Glu	Glu	Gly	Trp	Leu 605	Leu	Val	Ala
15	Leu	Asp 610	Tyr	Ser	Gln	Ile	Glu 615	Leu	Arg	Val	Leu	Ala 620	His	Leu	Ser	Gly
20	Asp 625	Glu	Asn	Leu	Ile	Arg 630	Val	Phe	Gln	Glu	Gly 635	Arg	Asp	Ile	His	Thr 640
	Glu	Thr	Ala	Ser	Trp 645		Phe	Gly	Val	Pro 650		Glu	Ala	Val	Asp 65	
25	Leu	Met	Arg	Arg 660	Ala	Ala	Lys	Thr	11e 665	Asn	Phe	Gly	Val	Leu 670	Tyr	Gly
30	Met	Ser	Ala 675	His	Arg	Leu	Ser	Gln 680	Glu	Leu	Ala	Ile	Pro 685	Tyr	Glu	Glu
50	Ala	Gln 690	Ala	Phe	Ile	Glu	Arg 695	Tyr	Phe	Gln	Ser	Phe 700	Pro	Lys	Val	Arg
35	Ala 705	Trp	Ile	Glu	Lys	Thr 710	Leu	Glu	Glu	Gly	Arg 715	Arg	Arg	Gly	Tyr	Val 720
	Glu	Thr	Leu	Phe	Gly 725	Arg	Arg	Arg	Tyr	Val 730	Pro	Asp	Leu	Glu	Ala 735	Arg
40	Val	Lys	Ser	Val 740	Arg	Glu	Ala	Ala	Glu 745	Arg	Met	Ala	Phe	Asn 750	Met	Pro
45	Val	Gln	Gly 755	Thr	Ala	Ala	Asp	Leu 760	Met	Lys	Leu	Ala	Met 765	Val	Lys	Leu
	Phe	Pro 770	Arg	Leu	Glu	Glu	Met 775	Gly	Ala	Arg	Met	Leu 780	Leu	Gln	Val	His
50	Asp 785	Glu	Leu	Val	Leu	Glu 790	Ala	Pro	Lys	Glu	Arg 795	Ala	Glu	Ala -	Val	Ala 800
	Arg	Leu	Ala	Lys	Glu 805	Val	Met	Glu	Gly	Val 810	Tyr	Pro	Leu	Ala	Val 815	Pro
55	Leu	Glu	Val	Glu 820	Val	Gly	Ile	Gly	Glu 825	Asp	Trp	Leu	Ser	Ala 830	Lys	Glu

(2) INFORMATION FOR SEQ ID NO::11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs (B) TYPE: nucleic acid

		(C) STRANDEDNESS: single (D) TOPOLOGY: linear	
_	(ii)	MOLECULE TYPE: cDNA	
5	(iii)	HYPOTHETICAL: NO	
10	(ix)	FEATURE: (A) NAME/KEY: - (B) LOCATION: 118 (D) OTHER INFORMATION: /note= "PCR reverse primer used for PUC18"	
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20	(2) INFO	RMATION FOR SEQ ID NO::12:	
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	(ii)	MOLECULE TYPE: cDNA	
30	(iii)	HYPOTHETICAL: NO	
35	(ix)	FEATURE: (A) NAME/KEY: - (B) LOCATION: 1115 (D) OTHER INFORMATION: /note= "PCR sequencing primer 628A used for pUC18"	
40	(xi)	SEQUENCE DESCRIPTION: SEQ ID NO::12:	
	CCCAAAGC	CA GGCCG	,15
A E	(2) INFO	RMATION FOR SEQ ID NO::13:	
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50	•	(C) STRANDEDNESS: double (D) TOPOLOGY: linear	
	(ii)	MOLECULE TYPE: cDNA	
55	(iii)	HYPOTHETICAL: NO	
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		·	

	CAGGICCUIG AGGGC	15
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10	(ii) MOLECULE TYPE: cDNA	
	(iii) HYPOTHETICAL: NO	
15 20	<pre>(ix) FEATURE: (A) NAME/KEY: - (B) LOCATION: 146 (D) OTHER INFORMATION: /note= "pUC18 - pLSM5 5' junction"</pre>	
20	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::14:	
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30	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 63 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
	(ii) MOLECULE TYPE: cDNA	
35	(iii) HYPOTHETICAL: NO	
40	<pre>(ix) FEATURE: (A) NAME/KEY: - (B) LOCATION: 163 (D) OTHER INFORMATION: /note= "pUC18 - pLSM5 3' sequence</pre>	
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::15:	
	CAAGGAGTGA GATTCTCTAG AGTCGACCTG CAGGCATGCA AGCTTGGCAC TGGCCGTCGT	60
50	TTT	63
-		

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What is claimed is:

1. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 934, 962, and 2535.

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2. A modified *Taq* DNA polymerase gene according to claim 1 wherein the altered nucleotide at position 89 is G, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A.

3.

claim 1.

4. Host cells transfected with the modified *Taq* DNA polymerase gene of

Host cells transfected with the modified Taq DNA polymerase gene of

claim 2.
 5. A modified Taq DNA polymerase essentially comprising the native Taq

DNA polymerase with an altered amino acid at positions 272 and 281.

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- 6. A modified Taq DNA polymerase according to claim 5 wherein the altered amino acid at position 272 is Ile and the altered amino acid at position 281 is Pro.
- 7. A modified Taq DNA polymerase gene essentially comprising the native Taq DNA polymerase gene having an altered nucleotide at positions 193 and 504.

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8. A modified *Taq* polymerase gene according to claim 7 wherein the altered nucleotide at position 193 is T and the altered nucleotide at position 504 is A.

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9. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 193, 504, 934, 962, and 2535.

10. A modified *Taq* polymerase gene according to claim 9 wherein the altered nucleotide at position 89 is G, the altered amino acid at position 193 is T, the altered amino acid at position 504 is A, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A.

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- 11. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 7.
 - 12. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 8.
 - 13. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 9.
 - 14. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 10.
 - 15. A modified Taq DNA polymerase essentially comprising the native Taq DNA polymerase with an altered amino acid at position 25.
 - 16. A modified Taq DNA polymerase according to claim 15 wherein the altered amino acid at position 25 is Cys.
 - 17. A modified Taq DNA polymerase essentially comprising the native Taq DNA polymerase with an altered amino acid at positions 25, 272, and 281

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- 18. A modified Taq DNA polymerase according to claim 17 wherein the altered amino acid at position 25 is Cys, the altered amino acid at position 272 is Ile, and the altered amino acid at position 281 is Pro.
- 19. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at position 341.
- 20. A modified Taq polymerase gene according to claim 19 wherein the altered nucleotide at position 193 is T and the altered nucleotide at position 504 is A.
- 21. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 341, 934, 962, and 2535.
- 22. A modified *Taq* polymerase gene according to claim 21 wherein the altered nucleotide at position 89 is G, the altered amino acid at position 341 is A, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A
- 23. Host cells transfected with the modified Taq DNA polymerase gene of claim 19.
- 24. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 20.
- 25. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 21.
- 26. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 22.

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- 27. A modified Taq DNA polymerase essentially comprising the native Taq DNA polymerase with an altered amino acid at position 74.
- 28. A modified Taq DNA polymerase according to claim 27 wherein the altered amino acid at position 74 is His.
- 29. A modified Taq DNA polymerase essentially comprising the native Taq DNA polymerase with an altered amino acid at positions 74, 272, and 281
- 30. A modified *Taq* DNA polymerase according to claim 29 wherein the altered amino acid at position 74 is His, the altered amino acid at position 272 is Ile, and the altered amino acid at position 281 is Pro.
- 31. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.
- 32. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.
- 33. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.
- 34. Polymerase chain reaction, wherein the improvement comprises use of the modified Taq DNA polymerase of claim 16.
- 35. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.
- 36. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18.
- 37. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.

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- 38. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 38.
- 39. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.
- 40. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.
- 41. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.
- 42. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.
- 43. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.
- 44. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 16.
- 45. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.
- 46. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18.
- 47. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.
- 48. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 28.

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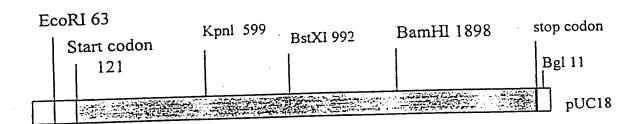
- 49. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.
- 50. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.
- 51. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.
- 52. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.
- 53. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.
- 54. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 16.
- 55. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.
- 56. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18
- 57. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.
- 58. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 28.
- 59. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.

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60. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.

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FIG. 1



Restriction map of gene for Taq DNA polymerase

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FIG. 2
Scheme for Zone Mutagenesis

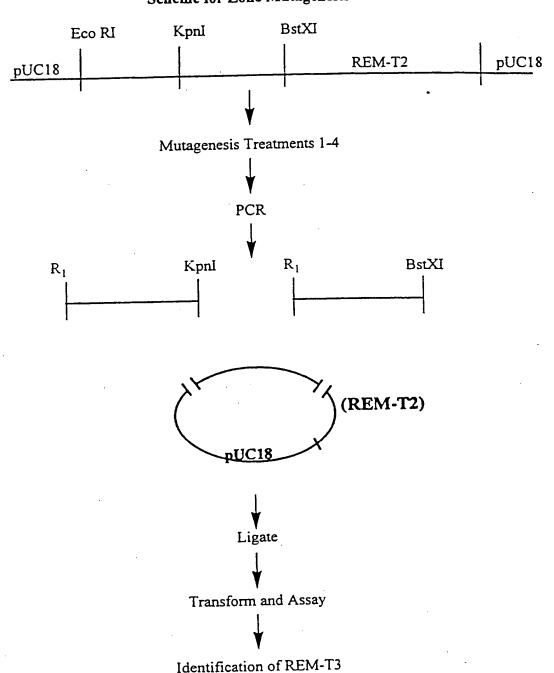
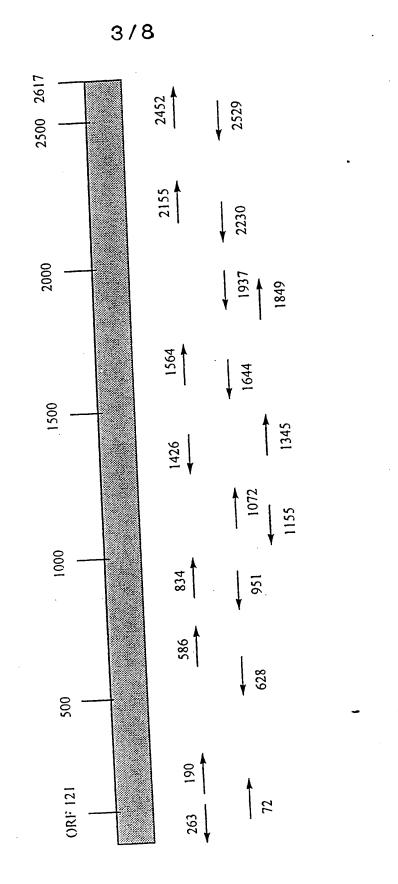
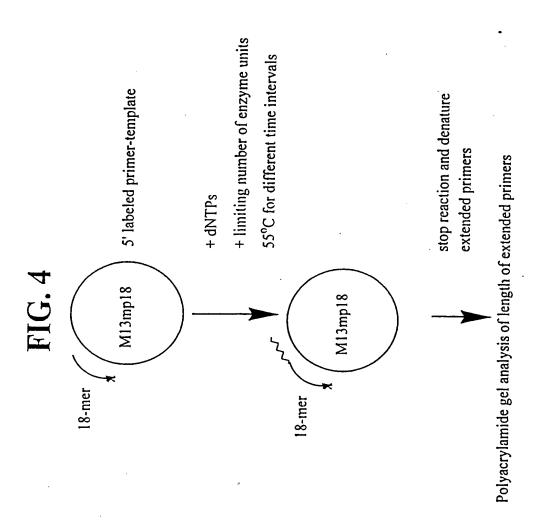


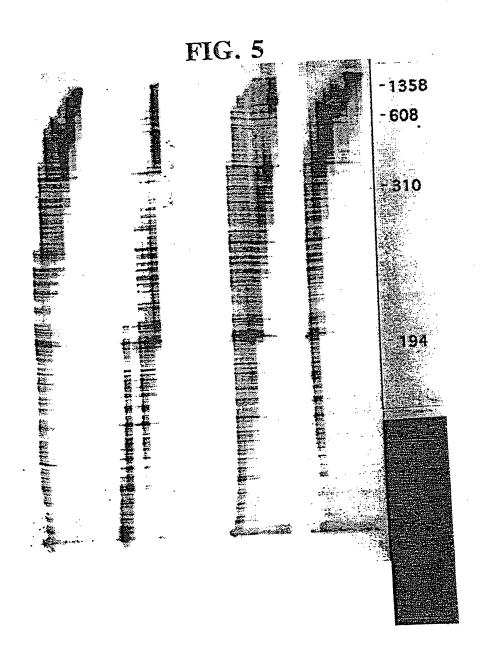
FIG. 3

Sequencing Primers for pLSM5



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FIG. 6

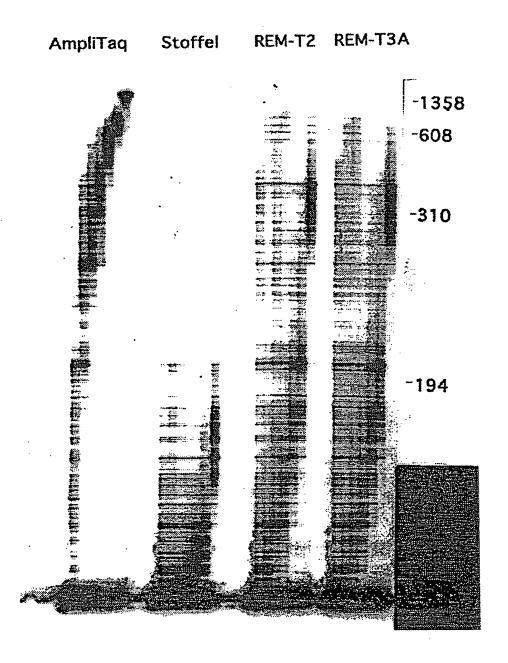
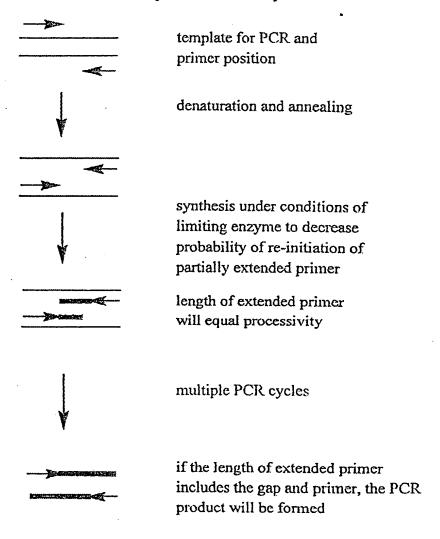


FIG. 7

PCR Analysis of Processivity



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FIG. 8

1 2 3 4 5 6 7 8

